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APPROVAL SHEET

Title of Dissertation: "Moderators of Coronary Vasomotion During Mental Stress in Coronary Artery Disease Patients: Stress Reactivity, Serum Lipoproteins, and Severity of Atherosclerosis"

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Doctor of Philosophy Degree
30 August 1996

Dissertation and Abstract Approved:

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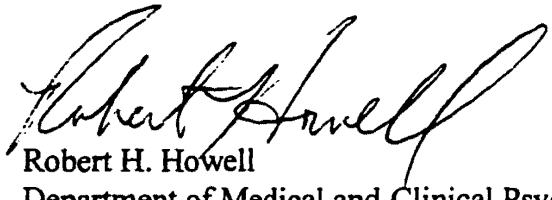

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ABSTRACT

Title of Dissertation: Moderators of Coronary Vasomotion During Mental Stress in Coronary Artery Disease Patients: Stress Reactivity, Serum Lipoproteins, and Severity of Atherosclerosis

Robert H. Howell, Doctor of Philosophy, 1996

Dissertation directed by: David S. Krantz, Ph.D.,
Professor, Department of Medical and Clinical Psychology

Impaired coronary artery vasomotion in response to behavioral triggers such as mental stress may be an important pathophysiological process involved in acute manifestations of coronary artery disease. The present research addresses the role of psychophysiologic responsiveness (reactivity), lipid levels, and atherosclerotic severity as moderators of coronary artery constriction / dilation in response to mental stress. Forty-five patients (39 males; mean age 59 years) undergoing diagnostic coronary angiography completed the study. Quantitative coronary angiography (QCA) was used to assess the diameter changes induced by mental stress and nitroglycerine administration. Changes in blood pressure, heart rate, and self-reported mood to mental stress, levels of serum LDL and HDL, and percent fixed stenosis at baseline (by QCA) were examined as moderators of vasomotion.

Results indicated that mental stress significantly increased blood pressure, heart rate, and self-reported distress. Contrary to predictions, atherosclerotic segments did not constrict more than angiographically smooth segments. Instead, results revealed specific moderators

of the coronary diameter response to mental stress in both atherosclerotic and smooth segments. In atherosclerotic coronary segments (n=33), stress reactivity but not coronary risk factors, lipoproteins, or severity of atherosclerosis moderated the coronary response to mental stress. Specifically, higher blood pressure reactivity was related to greater coronary vasoconstriction ($r=-0.42$, $p<.05$). In smooth coronary segments (n=45), hypertension independently moderated the coronary response to mental stress and stress reactivity (blood pressure and heart rate responses) interacted with LDL levels in predicting coronary vasomotion. Analyses among male subjects provided preliminary evidence that higher LDL levels were related to constriction to mental stress suggesting that, in general, coronary risk factors (e.g., hypertension, LDL) may play a greater role in smooth coronary segments as opposed to segments with angiographic atherosclerosis.

Possible study limitations are discussed including angiographic reliability and the magnitude of the constriction / dilation response. Given the possible clinical relevance of epicardial constriction to behavioral triggers such as mental stress, future research needs to examine what factors, in addition to the magnitude of reactivity, may help predict vasomotion in atherosclerotic segments.

Moderators of Coronary Vasomotion During Mental Stress in Coronary Artery Disease Patients: Stress Reactivity, Serum Lipoproteins, and Severity of Atherosclerosis

by

Robert H. Howell

Dissertation submitted to the Faculty of the Department of Medical and Clinical Psychology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1996

Dedication

This dissertation is dedicated to myself in the hopes that it may remind me that I must care for myself and feed my own soul first. Then and only then can I be peacefully aware and have the loving relationships that make life truly rich. I look forward to sharing in life with those I love.

While the effort of many produced this dissertation, it has remained a constant reminder of my successes and my failures. Hopefully it will become a reminder that both are acceptable. As my good friend John once told me "Anything worth doing is worth doing poorly". We are both still trying to live fully inside this idea.

Acknowledgements

Although, practically speaking, very few people may ever read this, it feels very pleasant to say out loud, in a seemingly public place, how thankful I am to so many people. In no particular order, other than the way they come to mind, I would like to thank everyone: The library staff, especially Greg, for 7 years of help; The faculty at USUHS, especially David, Andy and more recently Wijo; My dissertation committee: Dr. Krantz, Dr. Kop, Dr. Singer, and Dr. Goldstein; All the students and research assistants in Medical Psychology over the many years; My parents, for shaping my life (both the good and the bad) and for their support despite my meandering ways; and last, all of my friends over the course of graduate school (hopefully you know who you are and I will have thanked you in person, and if not, I hope I do so soon). I would also like to thank Vampire Bunny, a little-known superhero who's strength and guidance has been invaluable to this process, especially in the final few months.

A very special thanks goes to Elise, my one true love. You were with me through the worst of the dissertation process and I am very thankful that we had the chance to become closer while working through it. I am excited to spend the rest of my life with you.

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INTRODUCTION

Coronary artery disease (CAD) remains the primary cause of death in the industrialized world, claiming the lives of over half a million individuals each year in the United States alone (American Heart Association, 1992; Rutherford & Braunwald, 1992). Although coronary artery disease usually develops silently over many years, beginning in childhood with the insidious development of coronary atherosclerosis, onset of acute symptoms such as myocardial ischemia, myocardial infarction and sudden death generally occurs in mid to late adulthood. Within the last decade, the study of triggers of onset of acute clinical manifestations of CAD has become a focus of considerable research (Muller, Abela, Nesto & Tofler, 1994). In this regard, recent research provides support for the notion that mental stress and the associated physiological responses may be a trigger of acute disease manifestations (see Kamarck & Jennings, 1991; Krantz, Kop, Santiago & Gottdiener, 1996).

The present experiment is part of a larger study examining the role of cardiac supply and demand factors in myocardial ischemia, and the clinical significance of coronary endothelial dysfunction and mental stress. The research presented here examines impaired coronary artery vasomotion, recently emerging as an important pathophysiological process that may be involved in acute cardiac manifestations such as myocardial ischemia and myocardial infarction (Yeung, Khether, Ganz & Selwyn, 1992; Muller et al., 1994; Meredith Yeung, Weidinger, Anderson & Uehata 1993). More specifically the present research evaluates the role of hemodynamic and psychological stress reactivity, serum lipoprotein

levels (LDL and HDL), and the severity of atherosclerosis, in predicting coronary vasomotion during mental stress.

Research has shown that damage to the coronary endothelium impairs the ability of the coronary arteries to dilate, thereby allowing previously counteracted constrictive forces (e.g., α-mediated constriction during mental stress) to dominate once they are triggered. In healthy individuals with intact endothelium, mental stress leads to dilation of the epicardial coronary vessels. However in CAD patients, physiological reactivity associated with mental stress interacts with the pathophysiology of disease to trigger impaired vasodilation and possibly vasoconstriction. While it is known that the overall response of an arterial segment is the result of the interaction of vasoconstrictive forces and endothelial-mediated vasodilation (Furchgott & Zawadzki, 1980; Hodgson & Marshall, 1989; Griffith, Edwards, Lewis, Newby & Henderson, 1984), great variability exists within patient populations exhibiting documented atherosclerosis (e.g., Kuhn, Mohler, Satler, Reagen, Lu, & Rackley, 1991; Vita, Treasure, Yeung, Vekshtein, Fantasia, Fish et al., 1992) and relatively little is known about predicting the magnitude of disrupted vasomotion across patients. Therefore, based on current knowledge of the pathophysiology of coronary vasomotion, the present study examined the interaction of cardiovascular reactivity to stress with factors that are known to affect endothelial dysfunction: serum lipoproteins and severity of atherosclerosis. Evidence for the role of mental stress in CAD and for the clinical significance of coronary endothelial dysfunction is reviewed below. In addition, the role of psychological and hemodynamic responses to stress, serum lipoprotein levels, and severity of atherosclerosis on coronary endothelial dysfunction is reviewed to provide the rationale for the research

hypotheses.

Mental Stress and Clinical Manifestations of CAD

Physiological effects of stress. Over eighty years ago, Walter Cannon (1914; 1929) noted that emotional states such as fear and anger could elicit a massive sympathetic "fight or flight" response marked by increases in circulating catecholamines. Selye (1936; 1976) demonstrated a generalized stress response, involving both pituitary and adrenal cortical activation, to a variety of noxious stimuli. These two investigators provided the groundwork for the large body of accumulated research on mental stress and its physiological consequences. More contemporary stress research emphasizes the crucial role of interpretation or appraisal of stimuli and events for the perception of stress and the elicitation of physiological responses (see Lazarus, 1966; Mason, 1975). Accordingly stress will be defined in the present paper as a negative emotional state of the individual, dependent on interpretation or appraisal of threat, harm, or demand (Lazarus, 1966; see also Baum, 1990).

Stress is usually accompanied by a wide range of physiological responses including redirection of blood flow to active muscles and away from the gastrointestinal tract and kidneys, increased cellular metabolism, and increased release of glucose into the bloodstream (see Guyton, 1991). Stress-related autonomic arousal is also reflected in increased heart rate, heart contractility, cardiac output and blood pressure (see Krantz & Manuck, 1984), and a decrease in vagal or parasympathetic activation to the heart (Grossman & Sveback, 1987; Pagani, Mazzuero, Ferrari, Liberati & Cerutti, 1991). Hematological changes include

increased platelet activation and platelet aggregability (Patterson, Zakowski, Hall, Cohen, Wollman & Baum, 1994; Grignani, Soffiantino, Zuchella, Pacchiarini & Tacconi, 1991; Fitchett, Toth, Gilmore & Ehrman, 1983) as well as increased blood viscosity (Muldoon, Herbert, Patterson, Kameneva, Raible & Manuck, 1995; Patterson, Gottdiener, Hecht, Vargot & Krantz, 1993; Ehrly, Landgraf, Hessler & Saeger-Lorenz, 1988) leading to a state of hypercoagulation. Hormonal changes during stress include the release of epinephrine and corticosteroids from the adrenal medulla and adrenal cortex, respectively, as well as norepinephrine release from the adrenal medulla and sympathetic nerve terminals (see Glass, Krakoff, Contrada, Hilton & Kehoe 1980; Frankenhauser, 1975; Guyton, 1991).

There are two main subtypes of adrenergic receptors, α and β, each with two subtypes (i.e., α_1, α_2, β_1, β_2). While the subtypes of adrenergic receptors have differential effects throughout the body, the important physiological effects for the present proposal are those related to the coronary blood vessels and the cardiac tissue. α_1 receptors are postsynaptic mediators of constriction in blood vessels, including the coronary arteries. Current evidence suggests that postsynaptic α_2 receptors may also play a role in arterial constriction (Heusch, 1990) in addition to mediating the feedback inhibition of norepinephrine. Activation of the β_1 receptor elicits the cardiac effects such as increased contractility while the β_2 receptor mediates coronary dilation. Norepinephrine has its main effect on α-adrenergic receptors with only a slight effect on β-adrenergic receptors. Epinephrine, however, has approximately the same effect on both α- and β-adrenergic receptors (see Guyton, 1991).

Acute triggers of myocardial infarction and sudden death. In the United States alone, over half a million lives are lost each year to myocardial infarction and sudden cardiac death. As recently as the early 1980's, the prevailing clinical view was that acute syndromes such as myocardial infarction and sudden cardiac death were seen as random events, unpredictable and unrelated to patient behaviors. However, advances in our understanding of acute coronary artery disease such as the causal role of acute thrombosis and plaque disruption in unstable angina and myocardial infarction, and evidence that clinical events are more prevalent in the hours after awakening support the hypothesis that patient behaviors or transient physiological changes may act as acute triggers for the clinical manifestations of CAD (DeWood, Spores, Notske, Mouser, Burroughs, Golden et al., 1980; Davies & Thomas, 1984; Falk, 1983; Goldberg, Brady, Muller, Chen, Groot, Zonnefield et al., 1990; Muller, Stone, Turi, Rutherford, Czeisler, Parker et al., 1985). This research has made the study of the mechanisms of acute disease onset a focus of cardiovascular research (Muller, et al., 1994).

A model of acute coronary outcomes proposed by Muller et al. (1994) postulates that physical and mental activities of the patient elicit physiological changes that disrupt vulnerable plaque, thereby causing occlusive thrombus formation and acute myocardial infarction or sudden death (Muller et al., 1994). The model includes a trigger, a behavior or emotional state of the patient that produces acute physiologic changes such as arterial pressure surges or vasoconstriction. These physiologic or pathophysiologic changes are considered acute risk factors that may trigger clinical manifestations of CAD in the presence of vulnerable atherosclerotic plaque.

Stress as a trigger of clinical manifestations of coronary artery disease. A growing body of research now supports the hypothesis that mental stress can act as a trigger of myocardial infarction and sudden cardiac death (see Muller et al., 1994; Kamarck & Jennings, 1991; Markovitz & Matthews, 1991; Krantz et al., 1996, for reviews). Anecdotal reports and quasi-experimental data involving the health effects of natural disasters have suggested that mental stress may precede myocardial infarction and sudden cardiac death (Engel, 1971; Kark, Goldman & Lipstein, 1995; Trichopoulos, Katsouyanni, Zavitsanos, Tzonou & Dalla-Vorgia, 1983; Dobson, Alexander, Malcolm, Steele, & Miles, 1991). Epidemiological studies, including psychosocial prodromata preceding sudden cardiac death and myocardial infarction, the effects of emotional distress, vital exhaustion, and depression on the prognosis of post MI patients, and the role of severe life crisis such as loss of a spouse, also support the role of stress in the development of initial and secondary clinical CAD events (see Krantz et al., 1996; Carney, Freedland, Rich & Jaffe, 1995; Kamarck & Jennings, 1991; Kuller, 1978). In addition, the use of relevant animal models has given an increased understanding of the pathophysiology of myocardial infarction and sudden cardiac death at the basic science level. Advances in knowledge, such as the role of macrophages in unstable plaque formation and the role of fibrinolytic activity in myocardial infarction, will continue to help elucidate the possible mechanisms by which physiological reactions to mental stress may effect cardiac pathology (see Muller et al., 1994).

Despite the broad body of evidence gathered to date, scientific understanding of the role of psychological stress in CAD pathophysiology has been limited by reliance on clinical

disease manifestations that occur infrequently and cannot be ethically provoked in humans. Myocardial ischemia, a clinically relevant marker of coronary disease, is transient and reversible and allows for a better understanding of the relationships between stress and CAD. Myocardial ischemia results from an imbalance of myocardial oxygen supply and demand leading to a inadequate perfusion of the cardiac tissue, anaerobic metabolism, diminished or abnormal left ventricular contraction, electrophysiological changes, and sometimes, anginal pain (Rozanski, & Berman, 1987). The presence of ischemia confers significant prognostic risk of cardiac events for coronary artery disease patients, independent of coronary anatomy and degree of left ventricular impairment (The multicenter post-infarction research group, 1983; Weiner, Ryan, McCabe, Chaitman, Sheffield, Ferguson et al., 1984). Furthermore, the risk of future cardiac events increases dramatically with the magnitude of exercise-induced ischemia (Ladenheim, Pollack, Rozanski, Berman, Staniloff, Forrester et al., 1986), and the presence and extent of ambulatory ischemia out-of-hospital adds further risk (Rozanski & Berman, 1987; Modena, Corghi, Fantini & Mattioli, 1989; see Pepine, 1990 and Yeung, et al., 1992 for reviews). Given the clinical significance of myocardial ischemia, an understanding of the pathophysiology and triggers of ischemia may provide a link between the insidious development of atherosclerosis and the occurrence of life-threatening clinical events.

Using diary methodology and ambulatory ECG monitoring techniques, research has demonstrated that daily life myocardial ischemia frequently occurs during nonexertional activities, including mental stress (Barry, Selwyn, Nabel, Rocco, Campbell & Rebecca, 1988; Freeman, Nixon, Sallabank & Reaveley, 1987). Recent work by Krantz and

colleagues has corroborated the findings regarding the triggering role of mental stress in daily life ischemia (see Krantz, Gabbay, Hedges, Leach & Gottdiener, 1993 for review). Furthermore, laboratory studies have provided substantial evidence that mental stress tasks (e.g., mental arithmetic, public speaking) can cause myocardial ischemia in patients with CAD (e.g., Deanfield, Shea, Kensett, Horlock, Wilson, de Landsheere et al., 1984; Gottdiener et al., 1994; Rozanski, Bairey, Krantz, Friedman, Resser, Morel et al., 1988; Burg, Jain, Soufer, Kerns & Zaret, 1993). By comparing measures of ventricular function or perfusion during rest, mental stress, and physical exercise, these studies collectively have demonstrated that approximately 50% of patients with exercise-inducible ischemia also show ischemia triggered by mental stress (see Rozanski, Krantz, Klein & Gottdiener, 1991; Kamarck & Jennings, 1991; Krantz et al., 1996, for reviews).

Cardiovascular reactivity to stress as a CAD risk factor. Although the role of psychological stress as a trigger of CAD events has been supported by recent evidence, the role of the magnitude of the hemodynamic or emotional changes elicited during stress (reactivity) is less clear. Historically, researchers believed that the risk for CAD conferred by behavioral and psychosocial risk factors is mediated by the neuroendocrine and cardiovascular responses to psychological stressors (Jenkins, 1976; Krantz & Manuck, 1984). An underlying assumption of reactivity research is that, by measuring changes over resting levels produced by laboratory stressors, one can obtain an index of the state of the individual during challenges of everyday life and during exposure to environmental stressors. To the extent that reactivity effects disease processes, stable individual differences in the

magnitude of stress responses could have a corresponding effect on disease. In this regard, human studies investigating reactivity to mental stress have examined Type A behavior and hostility, gender, ethnicity, age, obesity, physical fitness, family history of hypertension (see Saab, 1989; Light, 1989; Anderson, 1989) as well as the idea that some individuals may be characteristically "hyperreactive" across time and situations (see Manuck, Kasprowicz, Monroe, Larkin & Kaplan, 1989).

Animal model studies using male and female cynomolgus monkeys fed an atherogenic diet have also supported the role of reactivity in the atherosclerotic process (Manuck, Kaplan, & Clarkson, 1983; Manuck, Kaplan, Adams & Clarkson, 1989). In these studies, animals showed large individual differences in heart rate response when exposed to a standard laboratory stressor (i.e., threat of capture). Results showed that at the end of the study, high heart rate reactors had nearly twice the amount of atherosclerosis as low heart rate reactors.

There is some epidemiological evidence that high reactors to stress are more prone to the development of clinical manifestations of CAD (Keys, Taylor, Blackburn, Brozek, Anderson & Simonson, 1971). In a prospective study examining reactivity to stress as a predictor of CAD, results from Keys et al. indicate that diastolic blood pressure to the cold pressor task was a significant predictor of CAD death and infarction at 20 year follow up. In a pilot study Manuck, Olsson, Hjemdahl & Rehnqvist (1992) examined the prognostic value of reactivity to a stressor in predicting reinfarction and / or stroke. Patients suffering from a new clinical event (39-64 months after stress testing) showed significantly greater systolic and diastolic blood pressure responses to the original stressor compared to those

patients remaining event-free. Evidence from field studies examining myocardial ischemia also suggest that high stress reactors (i.e., heart rate, rate-pressure product, diastolic blood pressure), in the laboratory, are more prone to ischemia during daily life (Blumenthal, Jiang, Waugh, Frid, Morris, Coleman et al., 1995; Krittayaphong, Light, Biles, Ballinger & Sheps, 1995). Results from Krittayaphong and colleagues (1995) reveal that peak heart rate response and heart rate change to a simulated public speech were significantly correlated with duration of ambulatory ischemia. Blumenthal et al, (1995), in larger sample of 132 patients, demonstrated that patients with ischemia during ambulatory monitoring showed significantly greater diastolic blood pressure, systolic blood pressure, and rate-pressure product during the laboratory stressor.

As reviewed, one current focus of CAD research is the role of stress as an acute trigger of the clinical manifestations of CAD. While the above research supports the notion that high reactors may be at risk for disease over time, little research has examined the role of reactivity as a trigger of acute diseases manifestations of CAD (e.g., myocardial infarction, sudden cardiac death) or a trigger of pathophysiological mediators of acute disease (e.g., ventricular fibrillation, plaque rupture, coronary vasoconstriction). Research examining myocardial ischemia in the laboratory does suggest that higher hemodynamic responses during a stressful task are more likely to elicit this clinical manifestation of CAD. Several studies have demonstrated that higher blood pressure responses to stress occur in patients with myocardial ischemia than those without evidence of stress-induced ischemia (Krantz, Helmers, Bairey-Merz, Nebel, Hedges & Rozanski, 1991; Blumenthal et al., 1995; Specchia, de Servi, Falcone, Gavazzi, Angoli, Bramucci et al., 1984; Stone, Krantz, McMahon,

Goldberg & Becker 1995). There is also some initial evidence that reactivity to mental stress and physical exercise may be related to vasomotion of the coronary arteries. This evidence is reviewed below and summarized in the section entitled Hemodynamic and psychological reactivity to stress.

Coronary Vasomotion

Clinical significance of coronary vasomotion. Traditional views of the pathophysiology of CAD and of myocardial ischemia held that a fixed or progressively worsening atherosclerotic plaque reduced flow such that acute demand increases on the heart (e.g., increased heart rate, increased contractility) elicited myocardial ischemia (see Cohn, 1992). However, recent research has supported the efficacy of vasodilating drugs on common ischemic syndromes, and provided evidence that daily-life ischemia is elicited at lower heart rates and rate pressure products compared to ischemia triggered by standard exercise tests (Deanfield, Shea, Riberio, Landsheere, Wilson, Horlock et al., 1984; Yeung & Selwyn, 1990). This research has rapidly changed the current view of the pathogenic role of transient vasoconstriction and supported the reasoning that a reduction in cardiac supply, possibly due to constriction of the epicardial coronary arteries, may be operative in "low-demand" ischemia (Maseri, Davies, Hackett & Kaski, 1990). In addition, recent studies examining endothelial dysfunction using coronary angiography have further elucidated and confirmed the role of vasoconstriction in variant angina (i.e., Prinzmetal's angina) (McFadden, Clarke, Davies, Kaski, Haider & Maseri, 1991; see Rutherford & Braunwald,

1992) and in unstable angina (Bogaty, Hackett, Davies & Maseri, 1994; Miwa, Fujita, Ejira & Sasayama, 1992).

While the specific clinical consequences of impaired endothelial function have not been clearly defined, coronary vasoconstriction is now seen as playing an important role in myocardial ischemia, and the restoration of endothelial function is thought to become an important clinical objective in controlling myocardial ischemia (Yeung, et al., 1992). Whereas the current study focuses on vasoconstriction or impaired dilation of the epicardial coronary arteries, it is important to recognize that most cases of myocardial ischemia are likely caused by a combination of factors including fixed lesions, platelet aggregation, vasoconstriction of the epicardial vessels, reduced dilation of the resistance vessels, and increases in myocardial demand (Cohn, 1992; Braunwald & Sobel, 1992; Rutherford & Braunwald, 1992).

The primary impetus for examining coronary vasoconstriction in the present research has been derived from work on myocardial ischemia. However, current research also suggests that coronary endothelial dysfunction may play a mediating role in other CAD endpoints such as myocardial infarction and sudden cardiac death (Muller, et al., 1994). Again, based on current evidence, myocardial infarction and sudden death may be triggered by physiological changes such as vasoconstrictive forces leading to plaque rupture. Research also suggests that lesion severity is not a good predictor of later infarction (Hacket, Verwilghen, Davies & Maseri, 1989). This has raised the possibility that the functional status of the coronary arteries, as opposed to anatomical status, may be important in predicting future cardiac events. A better understanding of the possible mechanisms

involved in the pathophysiology of myocardial ischemia and infarction, including coronary vasoconstriction, will be crucial to interventions aimed at decreasing the total number of deaths due to the clinical manifestations of coronary artery disease.

Physiology of epicardial coronary artery vasomotion. Until relatively recently the prevailing clinical view was that the coronary arteries were stable conduits for blood flow, similar to pipes, susceptible to narrowing only by way of fixed lesions. However, current research has begun to appreciate the dynamic nature of the coronary vessels, dilating and constricting to a variety of hemodynamic and neuroendocrine stimuli. The key to the dynamic nature of coronary vessels is the vascular endothelium, a single-celled lining of the inner surface of the blood vessel wall. Although historically the endothelial lining was viewed simply as a nonthrombogenic diffusion barrier and a site for the exchange of nutrients and metabolites, research during the last 15 years has revealed that the endothelium is an active secretory organ, producing potent vasodilators, vasoconstrictors, anticoagulant, and fibrinolytic substances (see Vanhoutte, 1988; Braunwald & Sobel, 1992; Mehta, 1995).

Central to the study proposed here is the release of endothelium-derived relaxing factor (EDRF), first recognized by Furchgott and Zawadzki (1980). Working with in vitro preparations of rabbit aorta, these researchers discovered that acetylcholine elicited arterial relaxation in the presence of the endothelium but contraction if the endothelium was damaged or absent. These findings were later replicated with in vivo animal preparations (Angus, Campbell, Cocks & Manderson 1983; Young & Vatner, 1987). Animal models and in vitro experiments have since shown that EDRF is nitric oxide, or a complex containing

it, and exerts its biochemical action through a pathway similar to nitroglycerine (Griffith et al., 1984; Palmer, Ashton & Moncada, 1987; see Mehta, 1995, for review). EDRF activates receptors on the smooth muscle cell that increase intra-cellular cyclic guanine monophosphate (cyclic GMP). This, in turn, triggers an increase in cyclic GMP dependent protein kinase that inhibits calcium release causing relaxation of the smooth muscle (see Mehta, 1995).

The use of animal models and in vitro experimentation with animal and human arterial preparations has also increased our knowledge concerning the release of EDRF and the disruption of endothelial function in pathophysiological states (see Meredith et al., 1993; Mehta, 1995; Busse, Mulsch, Fleming & Hecker 1993, for reviews). It is now recognized that EDRF release can be elicited by a variety of stimuli including acetylcholine, catecholamines, vasopressin, substance P, histamine, and platelet-derived factors such as serotonin, thrombin, and ADP (see Luscher, Richard, Tschudi, Yang & Boulanger, 1990; Busse et al, 1993; Meredith et al., 1993). While these stimuli elicit EDRF by way of receptors located on the endothelium, physical stimuli such as fluid shear stress, pulsatile stretching of the wall, and low arterial Po_2 also elicit the release of EDRF by way of the endothelium (see Busse et al., 1993). Both chemical and physical stimuli are said to be endothelium-dependent vasodilators due to the fact that an intact endothelium is needed for their dilatory effect. Basic research has shown that without a functional endothelium, stimuli such as aggregating platelets (through the release of serotonin and thromboxane A2) and catecholamines (through alpha-adrenergic receptors in smooth muscle) are potent vasoconstrictors (Toda, 1986; see also Braunwald & Sobel, 1992). However, an intact

endothelial lining will respond to these substances with the release of EDRF. EDRF, in turn, overrides the direct constriction and dilates the vessel (Cocks & Angus, 1983; Feigl, 1987). In addition, an intact endothelial lining provides a selective barrier to diffusion for substances acting on the smooth muscle cells, and inhibits the release and aids in the uptake of circulating norepinephrine and serotonin (Cohen & Weisbrod, 1988; Cohen, Zitnay, Weisbrod & Tesfamariam, 1988).

Animal models using pig, rabbit, dog, and monkey (see Meredith et al., 1993) and human experimentation in vitro (Forsterman, Mugge, Alheid, Haverich & Frolich, 1988; Chester, O'Neil, Moncada, Tadjkarimi & Yacoub, 1990) and in vivo (see below) have demonstrated that atherosclerotic damage to the endothelium disrupts the release or function of EDRF. The exact mechanism for this effect is unclear, but basic research suggests that endothelial damage can lead to decreased production of EDRF, decreased receptor-triggered release of EDRF, reduced penetration of EDRF into smooth muscle cells due to sub-endothelial intimal thickening found in atherosclerosis, biochemical inactivation of EDRF by superoxide free radicals present in atherosclerotic tissue, or some combination of these processes (see Boven, Jukema & Paoletti, 1994; Zeiher, Schachinger, Hohnloser, Saurbier & Just, 1994; Lamping, Piegors, Benzuly, Armstrong & Heistad, 1994; Meredith, et al., 1993; Mehta, 1995).

The most common research tool to assess the presence of endothelial dysfunction in humans is the intracoronary administration of acetylcholine. Most commonly, researchers compare coronary responses to acetylcholine, an endothelial-dependent vasodilator, with responses to nitroglycerine, an endothelial-independent vasodilator. A functional arterial

segment will dilate to acetylchloine administration. In turn, the presence of abnormal dilation to acetylcholine but appropriate dilation to nitroglycerine provides support that endothelial dysfunction is the underlying cause for the abnormal acetylcholine response. Ludmer, Selwyn, Shook, Wayne, Mudge, Alexander et al. (1986) were the first to examine the effects of acetylcholine administration in vivo in human coronary arteries. Results revealed that increasing concentrations of acetylcholine induced progressive constriction of atherosclerotic coronary arteries and dose-dependent dilation of healthy coronary segments. Since this first study by Ludmer et al, the presence of either impaired dilation or paradoxical vasoconstriction to acetylcholine has been demonstrated in CAD patients at coronary artery segments with severe and moderate atherosclerosis (Horio, Yasue, Okumaura, Takaoka, Matsuyama, Goto et al. 1988; Werns, Walton, Hsia, Sanz & Pitt, 1989; Yasue, Matsuyama, Okumura, Morikami & Ogawa, 1990). Impaired endothelial function can also occur in some patients with angiographically normal segments (Werns, et al., 1989; Vita, Treasure, Nabel, McLenachen, Fish, Yeung et al., 1990; Zeiher, Drexler, Wollschlager & Just, 1991; Yasue, et al., 1990). This impaired vasodilation or constriction in angiographically smooth segments seems to be restricted to patients with risk factors for coronary artery disease or with lesions in other segments or vessels (Vita et al., 1990; Reddy, Nair, Sheehan & Hodgson, 1994; El-Tamimi, Mansour, Wargovich, Hill, Kerensky, Conti et al., 1994) and seems to reflect the initial stages of atherosclerosis, undetectable by standard angiography.

As stated, studies of pre-atherosclerotic vessels indicate that risk factors may predict endothelial dysfunction because they predict the amount of early plaque or intimal thickening (Reddy et al., 1994). In this regard, a non-invasive measure of peripheral endothelial

dysfunction has been recently established using flow-mediated changes in arterial diameter in the forearm (Celermajer, Sorenson, Gooch, Spiegelhalter, Miller, Sullivan et al., 1992; Anderson, Uehata, Gerhard, Meredith, Knab, Delagrange et al., 1995). Current evidence suggests that this technique may provide a valuable non-invasive tool for assessing the early stages of atherosclerosis in young and at-risk individuals (Celermajer et al., 1992; see Anderson, Gerhard, Meredith, Charbonneau, Delagrange, Craeger et al., 1995). Initial evidence also indicates that, within a population exhibiting a range of moderate disease (e.g., from an absence of disease to mild atherosclerosis), peripheral endothelial function may help predict coronary endothelial responses to acetylcholine (Anderson, Uehata et al., 1995).

Human and animal model research have also increased knowledge concerning the restoration of endothelial function (Meredith et al, 1993). There is evidence that cholesterol reduction, administration of fish oil to the diet, L-arginine administration, and reduction of excess superoxide free radicals may enhance endothelial-dependent dilation (see Meredith, et al., 1993). Because these interventions increased endothelial functioning but did not affect atherosclerotic severity, this research suggests that endothelial function may be at least partly independent of atherosclerosis per se. Indeed, some risk factors such as cholesterol also seem to affect endothelial dysfunction independently of amount of atherosclerosis (see Total serum cholesterol, HDL, LDL).

In summary, research has revealed that damage to the endothelial lining of the coronary arteries, such as that occurring during atherosclerosis, disrupts the function and or release of potent vasodilators such as EDRF. Current research also suggests that dysfunction of the endothelium may play a crucial role in clinical manifestations of CAD. Animal model

research and research of isolated endothelium-dependent dilators such as acetylcholine have added greatly to knowledge concerning the mechanisms of endothelial dysfunction, including factors affecting endothelial restoration. However, there is currently a gap between the understanding of how specific stimuli effect coronary vasomotion in experimental conditions and how multiple factors interact in vivo with complex stimuli such as physical or mental stress (Wilson, 1990). The present study examines and quantifies these relationships. A brief overview of quantitative coronary angiography will be provided, after which human experimental evidence will be reviewed examining coronary vasomotion to behavioral triggers such as mental stress, cold pressor task, and physical exercise.

Measurement of coronary artery vasomotion using quantitative coronary angiography. In addition to its use in the clinical assessment of anatomical coronary artery disease, coronary angiography has been an invaluable tool in assessing coronary constriction and dilation (vasomotion), thereby increasing understanding of the role of coronary vasoconstriction in the disease process. A general overview is presented here and the reader is referred to the Methods section for exact procedure.

The use of coronary angiography to assess vasomotion of the epicardial coronary arteries involves a comparison of the coronary diameter before and after some manipulation (e.g., mental stress task, pharmacological intervention). The standard Judkins technique involves the use of a catheter attached to a three-stop cock manifold enabling the angiographer to switch rapidly between blood pressure monitoring, flushing the catheter with saline, and injecting the contrast medium (i.e., a non-ionic or ionic solution injected in order

to visualize the arteries under x-ray)(Levin & Gardiner, 1992). After entry into the femoral artery at the groin area, the guidewire/catheter combination is passed up to the descending thoracic aorta and into the left or right coronary ostium, the origination point of the left or right coronary arteries from the aorta. The catheter is then positioned and the camera angle is set for an optimal view of the lesion of interest (i.e., the arterial stenosis placed in the single, most severe, sharpest, and unforeshortened projection). Contrast agents are briefly injected directly into the coronary arteries and x-ray projections are recorded on 35 mm film for later quantitative analysis of coronary diameter.

While specific methods vary, the general procedure for quantitative coronary angiography involves the use of computer-aided edge detection and enhancement to measure coronary diameter while using catheter size as a measurement standard. This procedure generally allows for accurate assessment (i.e., measurement error 4-5%) of coronary diameter (Braunwald & Sobel, 1992).

All studies reviewed below used the standard Judkins approach for diagnostic catheterization and obtain the study views after diagnostic images are taken. Unless otherwise stated, all studies reviewed below titrated patients off anti-ischemic and vasoactive medications and performed a control infusion of intracoronary nitroglycerine after the pharmacological or task interventions and demonstrated the preserved vasodilation capacity of the smooth muscle. As stated standard experimental procedure involves nitroglycerine administration in order to demonstrate the ability of the arterial segment to dilate independently of the endothelium. This provides support that the any observed paradoxical constriction or impaired dilation seen during the task is an indication of endothelial

dysfunction per se.

Experimental Triggers of Coronary Vasomotion in Humans

Effects of mental stress. In order to examine the effects of mental stress on coronary epicardial vasomotion, Yeung, Vladimir, Krantz, Vita, Ryan, Ganz et al. (1991) used quantitative coronary angiography on 30 patients. Angiograms were taken after a resting baseline, 2-minute mental arithmetic stressor (n=26), after intracoronary nitroglycerine (n=26), and before and after intracoronary acetylcholine infusion (n=15). Four subjects served as non-stress controls and were non-stressfully instructed to count slowly upward by ones. Heart rate, blood pressure, and catecholamines were collected at baseline and post task. Two coronary segments were selected from each patient and were rated (blindly) as smooth, irregular or stenosed.

Results showed that mental stress elicited significant increases in heart rate (mean change 9 bpm), systolic blood pressure (mean change 12 mm/Hg), diastolic blood pressure (mean change 11 mm/Hg), and norepinephrine (mean change 40 pg/ml). The non-stress controls showed no changes in hemodynamic measures or in coronary vasomotion from rest to post-task. Collapsing across 52 segments, regardless of level of stenosis, coronary responses to mental stress ranged from 29% dilation to 38% constriction. Age was inversely correlated with response to mental stress with older patients demonstrating a tendency towards constriction (correlation coefficient not reported). Gender, total serum cholesterol levels, rate-pressure product, and change in norepinephrine were not significantly related to

coronary vasomotion. In the subset of 15 patients (27 arterial segments) exposed to acetylcholine, the correlation coefficient between vasomotion changes to mental stress and to acetylcholine was .47 and .58 for the lower and higher doses of acetylcholine respectively. Vasomotion responses also significantly differed across the three types of segments (i.e., smooth, irregular, stenosed). Mental stress elicited a mean constriction of 24% in the stenosed segments, 9% constriction in the irregular segments, and no significant change (3% dilation) in the smooth segments.

The results of this study support the hypothesis that damage to the endothelium is a predictor of vasomotion responses to mental stress. This is supported by the significant correlation between coronary responses to ACh and to mental stress. These results also provide evidence that vasomotion responses to mental stress differs between irregular and stenosed segments, with the most severely diseased segments showing the greatest constriction. Given the known endothelium dependent effects of ACh, the authors interpret the mental stress finding as reflecting that endothelial damage allows for alpha receptor mediated vasoconstriction unopposed by the normal EDRF release.

L'Abatte, Simonetti, Carpeggiani and Michelassi (1991) also examined the effects of mental stress on coronary epicardial vasomotion in 13 CAD patients. No pre-catheterization sedation was administered. Coronary angiograms, heart rate, and blood pressure were collected before and after mental stress testing. Mental stress consisted of 3 minutes of verbal subtraction (a two-digit number from a four-digit number) with the physician instructing the patient to concentrate and reproachment for lack of effort if incorrect. A comparison angiogram after infusion of nitroglycerine was not performed.

Results revealed that the stress task resulted in significant increases in heart rate (mean change 18 bpm) and systolic blood pressure (mean change 15 mm/Hg). For the group as a whole, the task elicited no significant change in coronary artery diameter (i.e., analyses collapsed across lesioned and smooth segments). Diameter changes ranges from 20% dilation to 20% constriction with mental stress, with most segments dilating or constricting less than 10%. The authors argue that this change is within their error of the measurement technique, including spontaneous changes, and therefore that no meaningful mean changes occurred due to the stress task. Separate analysis of stenosed segments also revealed no change in minimal luminal diameter. This also held for the two patients exhibiting ST-segment depression indicative of ischemia during mental arithmetic. While the hemodynamic changes are similar to those in Yeung et al. (1991), indicating at least similar magnitude of stress response, results of L'Abatte et al. (1991) revealed no overall constriction or constriction in the subset of stenosed segments. It is unclear if this is due to patient differences (e.g., severity of disease and/or endothelial dysfunction), differences in angiographic technique, or the relatively small sample size.

Boltwood, Taylor, Burke, Grogin, and Giacomini (1993) examined the effects of anger on coronary vasomotion in a small sample of coronary artery disease patients. Physiological analyses are based on a sample of 9 patients and all psychological analyses are based on 7 patients. Quantitative coronary angiography was assessed before and after an anger recall task in which the patient was prompted to describe in detail an anger provoking incident, including the emotional and physiological symptoms experienced at the time. Three segments, one lesioned and two non-lesioned, were chosen for analyses for each

subject. Heart rate and blood pressure were measured throughout the procedure. Anger, both before and after the task, was assessed with the State Anger Scale, a 10-item scale producing anger ratings between 10 and 40. Arousal was assessed using the Mehrabian-Russell Emotional Response Scale. In addition, trait measures of hostility and anger expression were obtained. A comparison measure with nitroglycerine was not done.

Results revealed a significant increase in anger and arousal but no significant increases in heart rate or blood pressure to the task. Quantitative coronary angiography revealed no overall significant change in arterial diameter from baseline to the anger task for either the lesioned or non-lesioned segments. However, a considerable range in diameter change was evident (34% dilation to 52% constriction). Correlational analysis revealed a significant correlation ($r = -.82$) between residualized anger scores and diameter change in the lesioned segment with higher anger scores related to constriction. Internal analyses revealed that true constriction only occurred with the three highest anger reports. A significant positive correlation ($r = .82$) was revealed between anger and the non-narrowed segment in one of the two clean segments examined. There were also strong but non-significant ($r's = -.60$ to $-.80$) correlations between diameter change in the lesioned segments and heart rate and blood pressure changes, with increased hemodynamic responses related to a tendency towards vasoconstriction. Anger-out expression style was the only trait measure significantly related to change in arterial diameter ($r = -.80$).

The study by Boltwood et al. presents some evidence that the magnitude of anger responses is related to diameter change in both smooth arterial segments (i.e., positive relationship) and lesioned segments (i.e., inverse relationship). The strong but non-

significant inverse correlations between hemodynamic changes and diameter changes in lesioned arteries deserve further attention. However, limitations of this study such as small sample size and the lack of an overall physiological reactivity effect make conclusions tentative.

Dakak, Quyyumi, Eisenhofer, Goldstein and Cannon (1995) examined the effects of mental stress on the microvasculature in patients (n=10) with LAD lesions less than 40% but with significant lesions in the LCX or RCA. These patients were compared to 5 patients without significant CAD. The mental stress consisted of 10 minutes of a video game with mild harassment. A comparison angiogram after nitroglycerine was not performed but 6 subjects received a repeat mental stress task with a prior injection of phentolamine (alpha antagonist). For the present summary, only results for epicardial vasomotion are discussed.

Results revealed that the stress task resulted in significant increases in heart rate (19 bpm, non-CAD patients; 12 bpm, CAD patients) and mean arterial pressure (13 mm/Hg, non CAD patients; 19 mm/Hg, CAD patients)($p<.01$). Coronary diameter of the LAD was measured in all patients at 0.5 cm distal to the tip of the doppler flow wire. At this location, there was no significant change in epicardial diameter to the stress (4 ± 10%, non-CAD; 1± 8%, CAD). However, at the point of the most severe occlusion in the patients with disease in the LAD, there was a mean constriction of 12 ± 8% to mental stress ($p<.01$).

In the subset of patients with repeat stress testing with phentolamine, epicardial constriction decreased (nonsignificantly) from 11% to 3%. Coronary resistance, reflecting microvasculature dilation, decreased significantly more with phentolamine than without ($p<.02$). The authors conclude that the microvasculature fails to dilate to mental stress in

patients with CAD (although coronary flow did not differ between the CAD and non CAD groups). The epicardial constriction, given that the lesions were less than 40%, was similar to that seen by Yeung et al. (1991).

Lacey, Contrada, Robbins, Tannenebaum, Moreya, et al. (1995) examined 11 patients (5 without CAD at angiography and 6 with atherosclerosis evidenced by angiography) who underwent 75 seconds of a simulated public speaking. This study examined multiple segments within each patient (n=58 segments; 11 subjects) and examined segments free of coronary disease (including luminal irregularities). No comparison angiography was obtained after nitroglycerine administration.

Mental stress produced a significant increase in heart rate (15 bpm), systolic blood pressure (28 mm/Hg), and diastolic blood pressure (11 mm/Hg) (all p's <.002). Analysis of coronary diameter changes revealed a significant overall constriction of 6.4 ± 1.6% ($p<.0002$) in all segments. There was no significant difference in vasomotion to mental stress between the segments from the 5 non CAD patients and the 6 diseased patients. The authors argue that the simulated speech task is a more potent stressor and this fact may account for the constriction seen in both non-CAD and CAD subjects.

In summary, studies of mental stress have revealed that stress can elicit both dilation and constriction in human coronary arteries and that the response to stress is similar to that of acetylcholine. There is at least some evidence that the response of a given segment varies with level of stenosis (i.e., stress eliciting no change in smooth arterial segments and constriction in stenosed segments, see Yeung et al., 1991). Evidence regarding reactivity per se is mixed. Yeung et al. (1991) show no relationship between hemodynamic or

neuroendocrine responses and vasomotion. However, in this study reactivity analyses appear to be collapsed across vessel type thereby obscuring the possible differential effects of reactivity depending on endothelial integrity. Boltwood et al. (1993) provide some evidence that hemodynamic and psychological reactivity are related to vasomotion. Separate correlations within vessel type revealed that higher reactivity was correlated with vasoconstriction in stenosed segments and correlated with vasodilation in smooth segments.

Effects of cold pressor. The effect of the cold pressor task on coronary vasoconstriction was examined in disease-free and coronary artery disease patients by Nabel, Ganz, Gordon, Alexander, and Selwyn (1988). Thirty patients recruited for diagnostic catheterization were categorized as normal (angiographically smooth coronary arteries and negative or equivocal exercise test), mild atherosclerosis (< 50% in at least one coronary artery), or advanced atherosclerosis (> 50% stenosis in at least one coronary artery). Multiple segments were examined in each vessel by quantitative coronary angiography. After a control period, patients submerged their hand and forearm into ice water for 90 seconds. Intracoronary infusion of nitroglycerine followed. Heart rate and blood pressure were measured continuously throughout the tasks. In a subset of patients, two separate cold pressor tasks were performed, the first drug-free (control) and the second after a 3-minute intracoronary infusion of propranolol, a beta-blocker.

Results revealed significant increases in heart rate (mean change = 11, 16, 14 bpm, respectively) and systolic blood pressure (mean change = 21, 28, 43 mm/Hg, respectively)

in all 3 groups. Angiography results revealed that the cold pressor task elicited a significant increase in coronary diameter in smooth segments (14% dilation), significant constriction in irregular segments (9%), and significant constriction in the stenotic segments (24%). Severity of atherosclerosis in the segment tested was more predictive of segment response than the overall disease status of the patient. This is reflected in the fact that irregular segments responded similarly in patients with mild and severe disease. Similarly, the response of smooth segments did not differ significantly (12%, 19%, and 8% dilation in clean, mild and diseased groups respectively) across the disease status of the patient. In the normal patients given intracoronary propranolol during a second cold pressor task, the dilation of normal segments was decreased by approximately 40% despite a similar hemodynamic response.

This study was the first to demonstrate that the normal response of coronary arteries to the cold pressor task is vasodilation. The authors interpret the paradoxical vasoconstriction of irregular and diseased arteries to the cold pressor as a reflection of endothelial dysfunction disrupting EDRF release and thereby allowing unopposed coronary constriction.

A second study examining coronary vasomotor responses to cold pressor (Zeiher, Drexler, Wollschlager, Saurbier & Just, 1989) also examined responses to acetylcholine infusion in a subset of the same patients in order to further examine the role of endothelial dysfunction in cold pressor responses. In this study, 87 subjects recruited for diagnostic catheterization were classified as patients with either clean coronary arteries and no risk factors for coronary artery disease (e.g., hypertension, hypercholesterolemia, diabetes)(group

1), or patients showing some angiographic evidence of coronary artery disease upon catheterization (group 2). All patients underwent a baseline period, then 90 seconds of hand and forearm immersion in ice water. A subset of 22 patients, 9 normal patients and 13 diseased patients, also underwent intracoronary acetylcholine infusion after the cold pressor task. Seventeen patients (11 normal and 8 diseased patients) were administered (intracoronary) the beta-blocker propranolol prior to administration of a second cold pressor task.

Results revealed a significant increase in heart rate (mean change of 8 and 9 bpm) and systolic blood pressure (mean change of 34 and 31 mm/Hg) for groups 1 and 2, respectively. In the 77 segments analyzed from the normal subjects the mean vasomotor response to the cold pressor task was 9% dilation. The smooth segments in the diseased subjects demonstrated a 7% constriction with only 15% of the smooth segments showing dilation. Irregular and stenotic segments from the disease subjects constricted 9% and 12 % respectively to the cold pressor task.

In the subset of patients tested with intracoronary acetylcholine, the responses of both normal and diseased arteries demonstrated rough correspondence to the cold pressor vasomotor response. Normal arteries, which dilated to cold pressor (mean dilation 13%), also dilated to acetylcholine (mean dilation 10%). Diseased segments, which constricted to the cold pressor (mean constriction 9%), also constricted to acetylcholine (mean constriction 23%). No correlations coefficients are provided to further examine the relationship of cold pressor to acetylcholine responses. The 17 patients receiving propranolol demonstrated no change in the cold pressor response in either normal or diseased segments.

Confirming results of Nabel et al. (1988), Zeiher et al. (1989) conclude that the normal response to the cold pressor of healthy coronary arteries is dilation. However, constriction appeared to be elicited at both early and late stages of atherosclerosis. Contrary to Nabel et al. (1988), the smooth segments of diseased patients tended to constrict. This supports more recent findings that acetylcholine and other endothelium dependent vasodilators may produce vasoconstriction in segments of patients with risk factors, and is seen as reflecting angiographically undetectable thickening of the intima occurring in the early stages of atherosclerosis.

Results also indicate that the response to the cold pressor was similar to acetylcholine, giving some support for the hypothesis that cold pressor constriction reflects damaged endothelium. The authors conclude from the propranolol finding that beta-blockade, leaving alpha-adrenergic mechanisms unopposed, did not significantly effect vasomotor responses in normal or atherosclerotic coronary arteries.

In a subsequent study, Zeiher et al. (1991) also compared the vasomotion effects of the cold pressor task to acetylcholine infusion (see Zeiher, et al., 1991 below, "Total serum cholesterol, HDL, LDL") across 4 groups: smooth coronary arteries with no risk factors, smooth coronary arteries with hypercholesterolemia, smooth study segment with diseased vessel elsewhere, and irregular segments (< 30% stenosis). Ninety seconds of hand and forearm immersion in ice water elicited a mean heart rate change of 10 bpm and a mean systolic blood pressure change of 18 mm/Hg with no significant differences across the 4 groups. The cold pressor task elicited dilation in patients with smooth arteries/no hypercholesterolemia and in patients with smooth arteries/ hypercholesterolemia (18% and

14% dilation, respectively). However, the cold pressor task elicited significant constriction in patients with smooth segments and lesions elsewhere (mean constriction 19%) and in patients with luminal irregularities (mean constriction 20%). Acetylcholine infusion appeared to be more sensitive to endothelial dysfunction causing significant constriction in groups 2, 3, and 4 (36%, 26%, 35% constriction, respectively, to the maximal dose of acetylcholine). That is, patients with smooth coronary arteries and no hypercholesterolemia were the only group not to constrict.

A recent study (Antony, Aptecar, Lerebours & Nitenburg, 1994) compared coronary vasomotion between hypertensive and normotensive patients with angiographically smooth segments to the cold pressor task. Three segments in each patient were examined by coronary angiography during baseline, cold pressor (120 seconds), and nitroglycerine. Results revealed that normal arteries in patients without hypertension dilated an average of 13% to the cold pressor. Normal arteries in hypertensive patients without other risk factors such as hypercholesterolemia, demonstrated a mean of 8% constriction to the cold pressor. Hemodynamic change scores were similar in both groups and comparable to previous cold pressor studies reviewed. The authors hypothesize that the coronary constriction seen in the hypertensive patients may be due to endothelial dysfunction related to early damage to the endothelium in hypertensive patients possibly causing heightened vascular reactivity to norepinephrine.

In summary, research has revealed that the cold pressor task elicits dilation of coronary arteries in the absence of atherosclerosis and risk factors for CAD. However, smooth coronary segments, in patients with either hypertension or atherosclerosis in vessels

other than the studied vessel, demonstrate paradoxical constriction during the cold pressor. There is some evidence that greater constriction occurs with more severe atherosclerosis (e.g., Nabel et al., 1988) but other studies have not supported this finding (e.g., Zeiher et al., 1989). Studies comparing the cold pressor task to acetylcholine infusion have revealed some correspondence in arterial responses between the two interventions, although acetylcholine may be more sensitive in detecting endothelial dysfunction in some groups (e.g., Zeiher et al., 1991). No data were available on the role of hemodynamic reactivity and arterial responses to the cold pressor task.

Effects of bicycle exercise. Several studies have assessed coronary vasomotion during bicycle exercise testing. The first (Gage, Hess, Murakami, Ritter, Grimm & Krayenbuehl, 1986), examined 12 stable coronary artery disease patients, without evidence of variant angina, before and after supine bicycle exercise (mean duration 3.5 minutes). An additional 6 patients were tested with intracoronary nitroglycerine prior to exercise. Normal coronary segments showed an average of 23% dilation with exercise. In the group receiving nitroglycerine, the normal segment dilated 21% to nitroglycerine and remained dilated an average of 29% during exercise. Stenosed segments, chosen as the most prominent or severe, constricted 29% during exercise. Nitroglycerine prior to exercise dilated the stenosed segment 22%. During exercise this dilation remained for the first 2 minutes then tended to decrease (14% mean dilation at peak exercise). There was an inverse correlation between change in mean arterial pressure and vasomotion in the stenosed segments ($r = -.63$), with higher blood pressure relating to greater constriction (calculated from study data, correlations

not presented in Gage et al., 1986). There was no significant correlation between mean arterial pressure and vasomotion in normal segments. There were no significant correlations between heart rate and vasomotion for stenosed or normal segments. The authors interpret the findings as reflecting an active vasoconstriction due to exercise-induced alpha-adrenergic stimulation or platelet activation in conjunction with endothelial dysfunction.

Gordon, Ganz, Nabel, Fish, Zebede, Mudge et al. (1989) examined 21 patients with coronary arteries ranging from smooth to > 50% stenosis in at least one vessel. Multiple segments were examined from each patient and categorized as smooth, irregular (less than 30% loss of lumen), or stenosed. Results showed that normal segments dilated to supine bicycle exercise an average of 14% with only 3 of the 21 segments constricting. Irregular segments constricted an average of 17% with only 3 of 16 segments not constricting. All stenotic segments constricted to exercise (mean of 23%). There were no significant correlations found between changes in rate pressure product, epinephrine, or norepinephrine, and vasomotor changes. However, it is unclear if the correlational analyses were performed collapsing across all segment types. In a subset of 6 patients, intracoronary acetylcholine was infused and coronary vasomotion reassessed. Acetylcholine and exercise elicited a parallel response, with segments that dilated to exercise also dilating to acetylcholine, and segments constricting to exercise also constricting to acetylcholine. No correlation coefficients were reported. The authors interpret these findings as indirect support for the role of endothelium in exercise-induced vasomotion.

The effect of intracoronary infusion of diltiazem, a calcium-channel blocker, on coronary vasomotion during bicycle exercise was examined by Nonogi, Hess, Ritter,

Bortone, Corin, Grimm et al. (1988) in 14 patients with positive exercise tests for ischemia (ST-segment depression > 1 mm) and angiographically defined stenosis (mean stenosis of 79%, range = 59% - 94%). Patients were exercised after a 4-minute infusion of intracoronary diltiazem. There was no comparison group given exercise without drug treatment. One stenotic and one smooth coronary segment were analyzed for each patient. Diltiazem elicited a 19% dilation in normal segments with peak exercise increasing this dilation to 24%. In stenotic segments, diltiazem dilated coronary segments an average of 11% and exercise elicited further dilation up to 23%. Although the mechanism for this effect is unclear the authors suggest that it is possible that diltiazem elicits a temporary restoration of endothelial function and EDRF-mediated dilation. In addition, the authors interpret the results as evidence that one mechanism of action for the clinical efficacy of calcium-channel blockers is its effect on vasomotion.

Two studies to date have examined the effects of beta-blockers on coronary vasomotion during bicycle exercise. Gaglione, Hess, Corin, Ritter, Grimm and Krayenbuehl (1987) compared the coronary responses to exercise in 12 previously studied control patients (see Gage et al., 1986) given no drugs prior to exercise with 10 patients given a relatively small dose of intra-coronary propranolol (1 mg). In normal coronary segments, propranolol increased coronary diameter 6% at rest and 13% at exercise compared with 23% dilation at exercise in control subjects. In stenosed segments, the vessels dilated to propranolol at rest (18%) and remained dilated at exercise (22%). Stenosed segments in the control group constricted an average of 29%.

Bortone, Hess, Gaglione, Suter, Nonogi, Grimm et al. (1990) also report evidence

that beta-blockade can inhibit coronary vasoconstriction induced in stenotic arteries by bicycle exercise. Eighteen control patients (i.e., exercised without beta-blockade) demonstrated 16% dilation in normal segments and 31% constriction in stenosed segments. These results are comparable to the results of Gage et al, (1986). Propranolol infusion (.1 mg/kg i.v.) over 4 minutes significantly reduced coronary diameter of both normal (24% constriction) and stenosed arteries (43% constriction) at rest. In patients given propranolol, exercise elicited dilation of smooth and stenosed coronary arteries back to pre-drug/pre-exercise levels. The mechanism for the protective effect of beta-blockers is unclear, specifically in light of evidence that beta-blockade may exacerbate spasm in patients with Printzmetal angina. The authors point out, however, that while nitroglycerine, calcium channel blockers, and beta-blockers may all attenuate coronary vasoconstriction of stenotic vessels to dynamic exercise, the mechanism for this effect is likely different for these three drugs (Bortone et al., 1990).

In summary, physical exercise elicits coronary vasodilation in segments without atherosclerosis and, on average, constriction in segment that are irregular or stenosed. Although correlation coefficients were not presented, there is some evidence that the coronary responses to exercise are similar to acetylcholine (Gordon et al., 1989). Studies examining physical exercise present no evidence that smooth arterial segments respond abnormally in patients with risk factors for CAD or disease in vessels other than the studied vessel. Results of Gage et al (1986) provide some evidence that greater blood pressure reactivity is related to greater vasoconstriction, but only in stenosed segments. Several studies examined the response to exercise after administration of anti-ischemic medications.

Results revealed that nitroglycerine, calcium-channel blockers, and beta-blockers all may counteract the vasoconstrictive effect of exercise seen in atherosclerotic segments, although the mechanism of effect may differ across these drugs. In addition, there is some evidence that calcium-channel blockers and beta-blockers seem to temporarily restore endothelial function. This is revealed by the fact that exercise elicited dilation in atherosclerotic arterial segments when drugs were administered prior to exercise. This coronary dilation to exercise given the presence of the drug was observed in the same segments that constricted to exercise prior to drug administration.

Effects of experimental increases in heart rate and of alpha-agonist infusion. To examine one possible mechanism of complex triggers (e.g., mental stress or exercise) and coronary vasoconstriction, Vita et al. (1992) examined the relationship between the coronary artery responses to acetylcholine and phenylephrine, an alpha-adrenergic agonist. Fifteen patients with < 30% stenosis (8 with completely smooth arteries) were referred for diagnostic catheterization. After completion of the diagnostic catheterization, 3 separate 2-minute infusions of acetylcholine (.14, 1.4, 14 micro grams/min) and 5 separate 2-minute infusions of phenylephrine (.016, .16, 1.6, 5.3, and 16 micro grams /min) were administered. Quantitative coronary angiography was assessed after each infusion dose for each drug and after infusion of intracoronary nitroglycerine. Two segments from the left anterior descending coronary artery were analyzed for each patient.

The 30 segments were classified as constricting (> 5% constriction) or non-constricting segments based on their response to the acetylcholine infusions. The mean

dilation response in the non-constricting group was 3% and the mean diameter change in the constriction group was 15%. Results from the phenylephrine infusion revealed that segments that constricted to acetylcholine also constricted significantly more to phenylephrine than the non-constricting segments. This was true across the 3 doses. High doses of phenylephrine constricted all segments, including segments that did and did not constrict to acetylcholine. Results revealed that significant constriction to phenylephrine occurred at a 100-fold lower dose in the acetylcholine constrictor group than the non-constrictor group. In addition, at the median concentration (i.e., 10^{-7} m for both drugs) there was a positive correlation ($r = .60$) between the responses to acetylcholine and to phenylephrine. There was no relationship of risk factors (e.g., hypertension, total cholesterol, family history, or past cigarette use) to the phenylephrine response. Results reveal that endothelial dysfunction, as measured by responses to acetylcholine, is associated with an increased vasoconstrictive sensitivity to an alpha-agonist. The authors conclude that the increased sensitivity to alpha-agonists, as opposed to increased release, may play a role in the paradoxical vasoconstriction elicited by mental stress, cold pressor and exercise.

In contrast to complex sympathetic psychophysiological stimulation such as mental stress, Nabel, Selwyn, and Ganz (1990) examined coronary vasomotion during isolated increases in heart rate induced via atrial pacing. Fifteen subjects were examined and divided into 3 groups: 1) 5 patients with smooth coronary arteries and no risk factors, 2) 5 patients with mild luminal irregularities in the study vessel, 3) and 5 patients who had > 70% lesions in at least one coronary artery. In the stenotic vessels, both a lesioned segment and a prestenotic segment were examined by quantitative coronary angiography. After the control

period, 4 separate 2-minute periods of atrial pacing were performed at 90, 110, 130, and 150 beats per minute. A comparison angiogram after infusion of nitroglycerine was also performed. ECG and blood pressure were recorded continuously and coronary angiograms were taken at the end of baseline and each 2-minute pacing procedure.

Results revealed a linear increase in control vessel diameter from 15% dilation at 90 beats per minute to a 30% dilation at 150 beats per minute. Patients with mild luminal irregularities showed no significant diameter change with 6% constriction at 90 beats per minute up to 12% constriction at the highest pace. In coronary segments with greater than 70% stenosis, the prestenotic segment significantly decreased in diameter at 90 beats per minute (26% constriction) and became progressively more constricted with higher levels of pacing (up to 52% at 150 beats per minute). The stenotic segment also constricted linearly to increased pacing with reductions in coronary diameter of 34% at 90 beats per minute up to 72% at 150 beats per minute.

The authors conclude that dilation to coronary pacing in the non-diseased segments reflects endothelial-dependent flow-mediated dilation. The intact endothelium is triggered by shear stress to release EDRF (see Pohl, Holtz, Busse & Bassenge 1986; Rubanyi, Romero, & Vanhoutte, 1986). The mechanism for coronary constriction due to pacing is less clear. While normal flow-mediated dilation is clearly impaired in groups 2 and 3, the mechanism for constriction in group 3 is unclear. While further research needs to examine the possible mechanism for the constrictive effect, it is evident from this research that an increase in heart rate alone can have a progressive constrictive effect on diseased coronary arteries.

As stated, the overall vasomotion of any arterial segment is based on the interaction of the stimulus (e.g., acetylcholine infusion, complex behavioral trigger such as exercise) and the integrity of the endothelial lining (i.e., ability to counteract direct constrictive effects with EDRF). Research reviewed above has focused on various triggers of vasomotion such as mental stress, cold pressor, and physical exercise. Reviewed below is research examining specific factors that affect endothelial function. Specifically, total cholesterol, LDL, HDL, and severity of atherosclerosis are examined with respect to their effect on endothelial function.

Factors Effecting Endothelial Function

Total serum cholesterol, HDL and LDL. Drexler & Zeiher (1991) examined the relationship of hypercholesterolemia to vasomotion in coronary arteries prior to the onset of angiographically visible coronary atherosclerosis. Patients were divided into three groups: 1) smooth arteries with no hypercholesterolemia (defined as total serum cholesterol ≤ 210 mg%) and no other standard risk factors, 2) smooth arteries with hypercholesterolemia, 3) luminal irregularities and hypercholesterolemia. Coronary vasomotion in the proximal left anterior descending artery was examined in these three groups before and after intracoronary infusion of 3 increasing concentrations of acetylcholine, as well as coronary flow increases triggered by papaverine infusion, and nitroglycerine infusion.

Results revealed that all 3 groups dilated to papaverine-increased flow, with group

3 showing significantly less dilation (mean dilation of 10%, 7%, and 5% in groups 1-3, respectively). Acetylcholine infusion produced consistent dilation in group 1. However, groups 2 and 3 demonstrated progressive constriction to the three doses of acetylcholine with 20% and 24% constriction, respectively, for groups 2 and 3 to the highest dose of acetylcholine. While groups 2 and 3 responded similarly to acetylcholine (i.e., constriction) compared to group 1 (i.e., dilation), group 3 tended to show greater vasoconstriction than group 2 at each dose of acetylcholine. The authors conclude that hypercholesterolemia is sufficient to cause endothelial dysfunction despite an absence of angiographically-determined atherosclerosis.

Zeiher, et al. (1991) also examined coronary vasomotion in 4 groups of patients: 1) smooth coronary arteries in patients with no risk factors for coronary artery disease (n=11), 2) smooth coronary arteries in patients with hypercholesterolemia (n=9), 3) smooth vessel segment investigated in patients with coronary lesions elsewhere (n=9), 4) luminal irregularities in the coronary segment examined (n=9). Coronary vasomotion was assessed in response to 3 interventions (i.e., flow-dependent dilation induced by papaverine, intracoronary acetylcholine infusion, and cold pressor task) in all 4 groups.

Results revealed that all groups except those with irregular lesions (group 4) maintained flow-mediated dilation (mean dilation of 22%, 15%, 20%, and 5% for groups 1-4 respectively). Results from the cold pressor task (reviewed above) revealed dilation of the coronary arteries in groups 1 and 2 but constriction in groups 3 and 4. Acetylcholine infusion elicited dilation in group 1 (i.e., smooth coronary arteries but no risk factors), but elicited comparable constriction across the remaining groups (36%, 26%, 35%, respectively).

It is suggested by the authors that the three tasks (flow-mediated dilation, cold pressor task and acetylcholine infusion) are able to detect progressively worse stages of endothelial dysfunction.

In order to examine the relationship between lipoproteins and coronary vasomotion, Kuhn et al. (1991) examined coronary responses to acetylcholine in 27 patients with lesions less than 50%. Both angiographically smooth (n=27) and angiographically irregular luminal segments less than 25% (n=14) were analyzed. Responses to the maximal dose of acetylcholine elicited predominately constriction in both smooth (mean constriction 13%) and irregular segments (mean constriction 19%). For both smooth and irregular segments, analyzed independently, vasomotion response correlated positively to HDL levels (r's = .60), with higher HDL levels being associated with less tendency to constrict. Total cholesterol, LDL and triglyceride levels were not correlated with vasomotion in this sample. Other risk factors, including hypertension, cigarette smoking, positive family history of coronary artery disease, and diabetes were also not correlated to vasomotion responses to acetylcholine.

In discussing the role of HDL on coronary vasomotion the authors point to clinical evidence for the beneficial effect of high levels of HDL for clinical events, especially in older populations. In addition, there is in vitro evidence that HDL levels promote endothelial proliferation and repair (Tauber, Cheng & Gospodarowicz, 1980) indicating a possible mechanism for the present results.

Vita et al. (1990) examined the relationship between standard risk factors for coronary artery disease and vasomotor responses to 3 separate concentrations of acetylcholine in patients with angiographically smooth coronary arteries. Results revealed

that total cholesterol levels and age were inversely related to vasomotion (r=.58 and .44, respectively). Both hypertension and family history of coronary artery disease, as dichotomous variables, were significantly related to vasomotion with greater constriction if positive. All 4 of these significant predictors of vasomotion produced an r^2=.63 in multiple regression analysis. Cigarette smoking, gender, blood pressure at catheterization, and coronary diameter response to nitroglycerine were all non significant. In addition, the total number of risk factors, analyzed as a continuous variable, was inversely correlated with vasomotion (r=-.73), with greater number of risk factors associated with vasoconstriction.

The results of this study show that in angiographically smooth coronary segments, risk factors predict endothelial dysfunction as detected by acetylcholine administration. However, it should be noted that the percent change in diameter size from rest to post acetylcholine was not used as the dependent variable in this study. Instead, all correlations and regression analyses, with the exception of a significant relationship with number of risk factors, used the slope of the dose response curve to the three concentrations of acetylcholine. It is unclear how this may affect the statistical analyses and affect comparisons with other studies. In addition, multiple segments of the LAD were analyzed by quantitative coronary angiography and only the segment with the greatest change from rest was used in analyses. This procedure may have decreased the variability in comparison with other studies, may have amplified the observed associations and therefore affect comparison of results.

Two recent studies provide evidence that total cholesterol levels or HDL and LDL subfractions are related to endothelial dysfunction and this relationship is independent of the

degree of atherosclerosis. Zeiher, et al. (1994) examined 26 patients with non-hemodynamically significant lesions (i.e., less than 30%) in the proximal LAD. Intracoronary ultrasound, a more precise measure than angiography for early stages of atherosclerosis, was used to establish the amount of intimal wall thickening. Both ultrasound-determined wall thickening and lipoprotein levels were related to vasomotion to 3 increasing concentrations of acetylcholine (3 separate 2-minute infusions). Results revealed that intimal wall thickening was inversely related to vasomotion ($r=-.82$) with greater thickening tending towards greater percent constriction. Multiple regression analysis demonstrated that HDL levels, LDL levels, and hypertension were independent predictors of vasomotion along with relative wall area. Age, gender, total cholesterol levels, and smoking were not independently related to vasomotion. After dividing patients at the 75th percentile for HDL levels it was further demonstrated that HDL was related to endothelial function independently of severity of atherosclerotic. That is, given similar levels of intimal wall thickening, as well as gender, age, hypertension, total cholesterol levels, LDL levels and smoking status, higher levels of HDL were associated with less constriction to all three acetylcholine concentrations.

Animal model studies and in vitro examinations suggest that the protective effect of HDL on endothelial function may be due its limiting effect on macrophage-derived superoxide anions and oxidized LDL, two potent inactivators of EDRF (see Zeiher et al., 1994). In addition, laboratory studies reveal that endothelial cell proliferation is increased in vitro by HDL (Tauber et al., 1980) and that LDL impairs endothelial function and this is reversible in the short term (Andrews, Bruckdorfer, Dunn & Jacobs, 1987; Tomita, Ezaki,

Miwa, Nakamura & Inoue, 1990).

Egashira, Hirooka, Kai, Sugimachi, Suzuki, Inou & Takeshita (1994) examined the effects of 6-8 months of pravastatin, a cholesterol lowering drug (HMG-CoA reductase inhibitor), on the endothelial response to acetylcholine in 9 patients. All patients had at least one lesion with greater than 75% stenosis and in a different vessel (the study vessel) a 25-40% stenosis. The pravastatin condition revealed a decrease in total serum cholesterol from 272 mg/dl to 187 mg/dl and in LDL from 195 mg/dl to 120 mg/dl. A significant attenuation of the constriction response was seen at the highest dose of acetylcholine when comparing baseline to post pravastatin (mean constriction of 21% and 11%, respectively). There was no significant change in the magnitude of the atherosclerotic lesion during the course of the study. Seven control patients received no cholesterol lowering medication over the 8 months. These control subjects demonstrated no change in serum cholesterol levels or in coronary artery responses to acetylcholine.

These results indicate that the effect of cholesterol on endothelial dysfunction is at least partly independent of atherosclerosis and that the beneficial effect of cholesterol-lowering medications may be due, in part, to improved endothelial function as opposed to regression of atherosclerotic lesions per se. This reasoning is supported by several cholesterol-lowering intervention trials. In general, these studies demonstrate a significant decrease in CAD morbidity and mortality with minimal effect on regression of atherosclerosis (see Meredith, et al., 1993, for discussion).

In summary, research has revealed that smooth coronary segments in patients with hypercholesterolemia will constrict to acetylcholine administration (Drexler & Zeiher, 1991;

Zeiher et al., 1991; Kuhn et al., 1991; Vita et al., 1990). However, dilation to cold pressor and to increases in flow seem to be maintained in this same population (Drexler & Zeiher, 1991; Zeiher et al., 1991). Kuhn et al. (1991) present evidence that HDL has a protective effect in both smooth and irregular coronary segments. Finally, existing evidence indicates that LDL and HDL subfractions of cholesterol may also affect endothelial dysfunction, and be at least partly independent of the magnitude of atherosclerosis (Zeiher et al., 1994; Egashira et al., 1994). However, the effects of LDL and HDL on coronary vasomotion has not been widely studied using complex triggers such as mental or physical stress, particularly in coronary segments with significant stenosis.

Severity of atherosclerotic lesion. As reviewed above, research has established that the initial stages of atherosclerosis involve functional changes in the coronary endothelial lining. It is thought that this is reflected in endothelial dysfunction as seen in response to endothelial-dependent dilators such as acetylcholine. Studies examining responses to acetylcholine in angiographically smooth coronary arteries reveal that patients with standard risk factors for CAD or with atherosclerosis in other vessels also exhibit endothelial dysfunction, compared to smooth segments in patients without risk factors. It has been presumed that risk factors are predictive of abnormal responses to acetylcholine because they reflect a greater level atherosclerosis not detectable by angiography. This view has been substantiated using intra-coronary ultrasound to measure atherosclerotic thickening (see Zeiher et al., 1994).

In addition, the majority of studies using acetylcholine as a measure of endothelial

dysfunction have revealed that coronary segments with luminal irregularities (atherosclerosis usually less than 30 %) show more consistent endothelial dysfunction than angiographically smooth segments (e.g., Drexler & Zeiher, 1991; Horio et al., 1988; Werns et al., 1989), although exceptions are present (Zeiher et al, 1991; Yasue et al., 1990). Segments with atherosclerosis $\geq$ 50% tend to display greater endothelial dysfunction than irregular segments with lesser disease, although appropriate statistical analyses are often inadequate or are not reported. Studies using mental stress, cold pressor, acetylcholine infusion, and heart rate pacing as stimuli have revealed that, on average, stenosed segments constrict to a greater extent than irregular segments (Yeung et al., 1991; Nabel et al., 1990; Nabel et al., 1988; Ludmer et al., 1986). Two studies, one using cold pressor and the other using both acetylcholine and serotonin infusions, present evidence that the average response of stenotic segments is similar to irregular segments (Zeiher et al., 1989; Vrints, Bult, Bosmans, Herman & Snoek, 1992). To date, no study has used quantitative coronary angiography in moderate and severe coronary disease patients in order to examine the relationship between severity of atherosclerosis and coronary responses to behavioral or pharmacological stimuli.

While the data do suggest that greater atherosclerosis is related to greater endothelial dysfunction, there is high variability of coronary responses with a similar extent of atherosclerosis, and it is possible for smooth segments within one artery to constrict more than adjacent segments with minor disease (e.g., Kuhn et al., 1991). This indicates that while magnitude of atherosclerosis is one factor that relates to endothelial function, endothelial function is not simply a reflection of severity of atherosclerosis. The use of the term "atherosclerosis" in the present proposal refers to anatomical lesion severity and does not

include any measure of the biochemical or histological changes that may be reflected in the atherosclerotic process. As stated above, the gross anatomical measurement of atherosclerotic severity is simply one variable (commonly used and frequently measured in coronary patients) that may be related to endothelial function.

Before turning to specific hypotheses and methods, a brief summary and overview of important conceptual issues is presented. An overview of evidence supporting the role of reactivity to stress as a predictor of coronary vasomotion is given and the rationale for the proposed interaction between reactivity and factors affecting endothelial function (i.e., severity of atherosclerosis, LDL, HDL) is discussed below.

Methodological and Conceptual Considerations

Hemodynamic and psychological reactivity to stress. As reviewed, evidence suggests that the magnitude of hemodynamic responses to stress (reactivity) may be related to the progression of atherosclerosis. In addition, greater reactivity is associated with increased frequency of myocardial ischemia in the laboratory and in daily life (e.g., Krantz et al., 1991; Blumenthal et al., 1995; Krittaphong et al., 1995, reviewed in present proposal). Prior research has demonstrated that acute psychological stress can cause coronary vasoconstriction (Yeung et al., 1991). Greater physiological and psychological responses by an individual may also be related to coronary vasomotion during tasks such as mental stress and exercise (Boltwood et al., 1993; Gage et al., 1986). Results of Gage et al. (1986) reveal that mean arterial pressure was correlated with constriction in diseased vessels but not

in smooth coronary segments. Results of Boltwood et al. (1993) provide preliminary evidence that increased hemodynamic and psychological reactivity correlate with constriction in diseased vessels and with dilation in normal vessels. One goal of the present proposal is to determine whether the magnitude of the stress response is predictive of vasomotion.

Current research suggest that both hemodynamic and emotional reactivity to mental stress may be associated with coronary diameter changes. Therefore, the present study examines both hemodynamic and psychological factors. Conceptually, the physiological and psychological indices of stress used in the present proposal are seen as markers of the stress response and are not viewed as direct mechanisms for the vasomotion of the coronary arteries.

An interaction model of stress and CAD. As prior research revealed, the effect of acetylcholine is dependent on the status of the endothelium, and the overall response of the arterial segment is seen as an interplay between direct cholinergic constriction and endothelium-dependent dilation (Furchgott & Zawadzki, 1980; Hodgson & Marshall, 1989). Similar to results from studies using acetylcholine, research examining complex triggers such as mental stress suggest that the effect of a given trigger depends on the functional integrity of the endothelium. That is, the specific stimulus under investigation, whether acetylcholine or general sympathetic activation, interacts with the functional integrity of the endothelium to produce constriction or dilation. In this regard, acetylcholine elicits dose response dilation of coronary segments with intact endothelial function and dose response constriction in

arterial segments with endothelial dysfunction (Ludmer et al., 1986). In- vitro animal preparations reveal this dichotomous response also occurs in response to platelets (Houston, Shepherd & Van Houtte, 1986). Nabel et al (1990) reveal that heart rate increases in humans are associated with linear increases in either dilation or constriction, depending on endothelial status. The differential response to increasing heart rate depending on the atherosclerotic severity is shown in Figure 1. Figure 1 presents data replotted from results presented in Nabel et al, 1990. As shown, increases in heart rate elicit a "dose-dependent" dilation in smooth coronary arteries, produce little effect in irregular arteries, and elicits progressive constriction in stenosed coronary arteries. It is hypothesized in the present study that this interactional model will also apply to the effect of mental stress reactivity on coronary vasomotion. As reviewed, results from a mental stress study (Boltwood et al., 1993) reveal a relationship between psychological reactivity and dilation in smooth segments and a relationship between reactivity and constriction in atherosclerotic segments. With the exception of this preliminary evidence, no study has specifically examined the interaction of reactivity to mental stress with other factors (e.g., severity of atherosclerosis, LDL, HDL) in predicting coronary vasomotion.

The rationale for hypothesizing an interactional model of reactivity and endothelial dysfunction in the prediction of coronary vasomotion is based on the biochemistry and pathophysiology of endothelial function. It is of theoretical interest to note that psychological studies have traditionally analyzed the independent effects of mental stress reactivity on cardiovascular disease. Recent evidence suggests that examining interactions between reactivity and other risk factors (e.g., hypertension, smoking) or reactivity and

underlying disease status may be useful. For example, stress reactivity seems to be related to left ventricular mass, but only among hypertensives (Matthews, Markovic & Bunker, 1995). In addition, there is evidence that the relationship between reactivity to stress and severity of atherosclerosis holds mainly for smokers (Kaplan, Cohen, Salonen, Salonen, & Kauhanen, 1995). The present study assessed the interaction of stress reactivity with a well established CAD risk factor (i.e., serum levels of LDL and HDL) and with the underlying severity of disease (i.e., atherosclerotic severity) in predicting the effect of mental stress on coronary vasomotion.

Summary and Rationale

Current research suggests that, as a result of impaired endothelial function, abnormal epicardial vasomotion may be a mediator of clinical manifestations of CAD such as myocardial ischemia and myocardial infarction. Research reviewed above has also revealed that impaired vasodilation or paradoxical vasoconstriction in coronary arteries may be elicited by mental and physical stressors, suggesting that cardiac supply factors may be an important mechanism linking mental stress and clinical manifestations of CAD. Despite advances in scientific understanding of endothelial dysfunction during the past decade, relatively little research has examined predictors of vasomotion to behavioral triggers such as mental stress. In addition, little is known concerning the role of responsiveness (reactivity) to stress, particularly as it interacts with other risk factors and underlying severity of disease. Therefore, the purpose of the present study was to examine specific predictors

of epicardial coronary diameter changes to mental stress: 1) severity of atherosclerosis, 2) serum levels of LDL and HDL, and 3) hemodynamic and psychological reactivity to mental stress.

HYPOTHESES

Hypotheses for Atherosclerotic Coronary Segments:

1. Severity of atherosclerosis (as measured by quantitative coronary angiography) will predict changes in coronary diameter in response to stress such that greater severity of disease will be associated with a greater tendency towards constriction from rest to mental stress.

2. Magnitude of hemodynamic and psychological reactivity (responsiveness) to stress (heart rate, systolic and diastolic blood pressure, self-reported stress) will predict coronary vasomotion, with greater stress reactivity being associated with greater coronary constriction.

3. Serum levels of LDL and HDL will predict mental-stress induced coronary vasomotion, such that higher levels of LDL will be associated with greater constriction and higher levels of HDL will be associated with less constriction in

response to stress.

4. The magnitude of reactivity to stress will interact with severity of atherosclerosis and serum lipoprotein levels. Given indicators of greater endothelial dysfunction (i.e., higher LDL, lower HDL, more severe atherosclerosis), higher reactivity will be associated with a greater tendency towards coronary constriction. However, given the presence of factors indicative of less endothelial dysfunction (i.e., lower LDL, higher HDL, less severe atherosclerosis) higher reactivity will be associated with less coronary constriction.

Specifically, two separate 2-way interactions are hypothesized: 1) Stress reactivity will interact with severity of atherosclerosis in predicting coronary responses to mental stress; and 2) Stress reactivity will interact with serum lipoprotein levels in predicting coronary responses to mental stress.

Hypotheses for (angiographically Smooth) Non-atherosclerotic Coronary Segments:

5. Magnitude of reactivity (responsiveness) to stress (heart rate, systolic and diastolic blood pressure, self-reported stress) will predict coronary vasomotion. In contrast to atherosclerotic segments, greater stress reactivity will be associated with greater coronary dilation in response to mental stress.

6. Serum levels of LDL and HDL will predict mental-stress induced coronary

vasomotion, such that higher levels of LDL will be associated with greater constriction (or less dilation) and higher levels of HDL will be associated with greater dilation (or less constriction) in response to stress.

7. Similar to hypotheses for lesioned coronary segments, there will be an interaction of stress reactivity with factors affecting the functional integrity of the endothelium (i.e., LDL, HDL). Specifically, a 2-way interaction is hypothesized with stress reactivity interacting with serum lipoprotein levels in predicting coronary vasomotion to mental stress.

METHODS

Design and Overview

The present study examined several predictors of the response of the epicardial coronary arteries to mental stress in patients undergoing diagnostic coronary angiography. After the diagnostic catheterization, a brief mental stress task (serial subtraction of 7's with harassment) was administered immediately after the resting baseline. The dependent variable is the percent diameter change, in angiographically normal and lesioned coronary segments, from rest to mental stress. The predictor variables are severity of atherosclerosis, measured by quantitative coronary angiography, resting levels of LDL and HDL cholesterol subfractions, and hemodynamic and psychological responses during mental stress.

Subjects

Fifty-one patients were recruited at the Washington Department of Veterans' Affair Medical Center and Walter Reed Army Medical Center as part of larger, NIH-funded study examining the effects of cardiac supply and demand factors on myocardial ischemia. Six patients were excluded prior to analyses due to technically inadequate angiography films leaving a total of 45 patients for analyses. Patients referred for diagnostic cardiac catheterization for the evaluation of angina syndromes or determination of the extent of coronary artery disease were recruited into the study. Patients with unstable angina, recent myocardial infarction (< 1 month), known ejection fraction less than 30% prior to catheterization, severe valvular disease, severe peripheral disease, congestive heart failure, or patients over the age of 80 were excluded prior to recruitment. Patients were also excluded if it was contraindicated to be titrated off all anti-ischemic medications or if they had a recent negative diagnostic exercise tolerance test. Additional exclusion criteria applied after the diagnostic catheterization included left main disease and severe proximal triple vessel disease. Patients were included in the present analyses if the coronary arteries were found to be free of disease (n=11) at catheterization. Informed consent was obtained in accordance with the requirements of the Veterans Administration Hospital, Washington D.C., the Walter Reed Army Medical Center and the Uniformed Services University of the Health Sciences Human Use Committees.

Procedure

Vasoactive and anti-ischemic medications including nitrates, calcium-channel blockers, beta-blockers, and angiotensin-converting-enzyme inhibitors were withheld 24 hours prior to catheterization. Long-acting beta-blockers were withheld 48 hours prior to catheterization. If the beta-blocker dose was too high for abrupt discontinuation, dosage was 50% at 72 hours prior to catheterization and 0% at 48 hours. Patients were allowed to use sublingual nitroglycerine, if necessary, up to the morning of the catheterization.

Prior to any medications, a 10-cc blood sample was drawn for assay of lipid profile (see below). Standard pre-operative sedation (diazepam) was withheld and replaced, only if deemed necessary for adequate diagnostic catheterization, with Versed (0.5 mg) immediately prior to the procedure. Patients received anticoagulation with 2000 U heparin. Diagnostic catheterization of the right and left heart was performed by the standard Judkins technique with right femoral approach. After the completion of the diagnostic catheterization, an additional 3000-5000 U of heparin was given intravenously and the baseline period was started. Throughout the study, heart rate, intra-coronary blood pressure, and an electrocardiogram were recorded continuously. Mood was assessed after the rest period and after the mental stress task using 7-point Likert scales. A coronary angiogram was taken at three time points 1) at the end of the rest period, 2) approximately 1.5 minutes into the mental stress task, and 3) 30 seconds following infusion of nitroglycerine.

As part of the larger study, a subset of patients (n=5) received an acetylcholine infusion prior to mental-stress testing. A 10^{-5} dextrose solution of acetylcholine was infused with the use of an infusion pump (Medex Inc., Medfusion Model 2001, Deluth, Georgia) at 1.6 ml per minute for 3.0 minutes. For this subset of patients the 3-minute rest period prior

to mental stress was initiated after the acetylcholine infusion and coronary angiogram.

Mental Stress Testing

The rest period was initiated with the room lights turned down, the ambient noise reduced to the lowest possible level, and the patient reassured and encouraged to relax during the next several minutes. After the rest period the lights were turned on and an investigator administered the baseline period self-report questions. Immediately after the self-report measures were taken the patients listened to a tape recording instructing them to count backwards from a 4 digit number, by 7's, as quickly and as accurately as possible. During the task (1.5 minute duration), the patient was intentionally frustrated by the investigator by being corrected and told to perform more quickly. In addition, a recorded metronome is played through the tape recorder in order to further frustrate the patient and increase awareness of time pressure. At 1.5 minutes, the post mental stress coronary angiography was taken. The patient was administered the post-task self-report questions and reassured that they performed well. Intracoronary nitroglycerine (200 microgram bolus) was administered after the cessation of the task and a final angiogram was taken in order to assess the integrity of endothelium-independent dilation.

Demographic and Clinical Variables and Risk Factors

Age, weight, smoking history, and family history of CAD were assessed during the

intake interview. Hypertension (i.e., a dichotomous variable with previously diagnosed and/or treated hypertension or current resting blood pressure > 140/90 deemed positive) and functional angina class (NYHA) were assessed during the intake interview and physical examination. Number of diseased vessels was assessed during the diagnostic catheterization.

Low Density and High Density Lipoproteins

LDL and HDL were determined by the VA Medical Center Lipid Laboratory or the Walter Reed Army Medical Center Lipid Laboratory. With the patient in a fasting state, a blood sample was obtained from the femoral sheath prior to the administration of heparin at the time of catheterization. At the VA site, total cholesterol and triglycerides were determined by an endpoint method using a commercially available reagent system and analyzer (Boehringer Mannheim Corporation, Indianapolis, IN). HDL was determined using the same method as total cholesterol following the precipitation and centrifugation of LDL and VLDL. Fractionation was performed with magnesium/phosphotungstate according to a modification of the Burnstein and Samaille method. LDL was calculated using the Friedewald equation:

$$\text{TChol (mg/dl)} - [(\text{trig (mg/dl)} / 5) + \text{HDL (mg/dl)}]$$

At the Walter Reed site, total cholesterol and triglycerides were determined by an endpoint method using a commercially available reagent system and analyzer (Kodak Ektachem, dry slide technology). HDL was determined using the same method as total cholesterol following the precipitation and centrifugation of LDL and VLDL. Fractionation

was performed with phosphotungstate in conjunction with Magnesium Chloride. LDL was calculated using the Friedewald equation (see above).

Response to Mental Stress

Blood pressure and heart rate. Arterial (aortic) blood pressure measures from the catheter tip were continuously recorded throughout the task and rest periods using a Midas system 2000 (E for M corporation, Lenexa, KS). Blood pressure recordings were hand-edited for artifact and the average blood pressure calculated for the 3 minutes of the rest period and the entire stress task (from start of patient counting to immediately prior to the angiogram). ECG was recorded continuously throughout all the procedures. Mean heart rate was calculated from the ECG for rest period and stressor.

Self-reported stress. Self-reports of anger, anxiety, frustration, irritation, and chest pain were verbally rated on a 7-point Likert scale (1 = Not at all, 7 = Very much). These measures were taken at the end of the rest period and immediately following the math stressor.

Measurement of Coronary Diameter

After the diagnostic catheterization, the cineangiographic system (Bicor, Siemens-Elema, California) was set to position the selected arterial segment in the center of the field

of view and at a single position in space (Yeung, et al., 1991; Ludmer et al., 1986). The angle of the angiographic view was kept constant between the rest and task angiograms. After the rest period and at 1.5 minute into the mental stress task, coronary angiography was performed using a single 5-7 cc bolus of non-ionic contrast medium (Isovue-370, Squibb Diagnostics, Princeton NJ) hand-injected into the left or right coronary artery, depending on the study vessel.

Quantitative coronary angiography (QCA) data were analyzed at the Washington Hospital Center Angiographic Core Laboratory. QCA technicians and readers were blinded to patient characteristics, lipoprotein levels, stress reactivity, and hypotheses concerning constriction and dilation of individual segments. Cineframes demonstrating the arterial stenosis in the single, most severe, sharpest, and unforeshortened projection were selected for quantitative angiographic analysis. Cineangiographic frames were matched for their location within the cardiac cycle for sequential measurements. Cineframes were optically magnified 2:1 to 2.5:1 and digitized using a cine-video converter. An automated edge-detection algorithm (CMS, MEDIS, The Netherlands) was applied to the digitized image using a validated algorithm (Koning, van der Zwet, Von Land & Reiber, 1992; Reiber, van der Zwet, von Land, Konig, Loois, Zorn et al., 1989; van der Zwet, Pinto, Serruys, & Reiber, 1990). Specific features of the CMS include two point user-defined pathline (centerline) identification, arterial edge detection using a 50% weighted threshold between the first and second derivative extrema, and an arterial contour detection using a minimal cost matrix algorithm. Using the contrast-filled injection catheter as the calibration source, absolute measurements of the reference and minimal lumen diameter were obtained (in mm). Percent

stenosis was calculated as the percent narrowing of the artery diameter at the point of minimal narrowing. Calibration factors range from 0.08 to 0.10 mm per pixel. "User-defined" vessel diameters were used to obtain average arterial dimensions 10 mm proximal and distal to the lesion. An "interpolated" vessel diameter was used to obtain an estimate of the "normal" arterial dimension at the site of the minimal lumen diameter. The identical reference segment region was measured after resting baseline and after each experimental intervention. Based upon these measurements, percent diameter stenoses were determined. Similar measurements were made for a normal or nondiseased arterial segment. Repeated analyses of reference and minimal lumen diameters using the CMS system have demonstrated variabilities of 0.12-0.18 mm and 0.09-0.16 mm; reproducibilities are 3.7-5.8% for percent diameter stenosis (Lesperance, et al., 1992).

The main dependent variable in the present analyses is the percent change in the mean diameter across the lesion or across the entire control segment from rest to mental stress. Values are presented as percent change with negative values representing vasoconstriction and positive values representing dilation. Because of greater reliability the present study examines the mean diameter change across the entire length of the lesion or smooth segment instead of examining the change in minimal luminal diameter.

Power Analysis

For each hypothesis, the estimated sample size needed to detect a significant effect was determined given $\alpha = .05$ and power $(1-\beta) = 0.80$. The Model II error was used in

calculating the estimated sample size (n^*) for an *F* test on sR^2 (i.e., variance added to the total R^2 by the interaction with the variance partialled for the covariates and independent predictors)(see Cohen & Cohen, 1983, p.159 for discussion of Model I and Model II error). Power was calculated for the interaction hypotheses below.

The estimated effect sizes were based on empirical data presented in the present proposal and general guidelines concerning power analysis for the behavioral sciences (see Cohen & Cohen, 1983). In general, an approximate r^2 of 0.10 - 0.12 corresponding to a correlation coefficient (r) of 0.31 - 0.35 is estimated for the independent predictors and the interaction effects. Given these effects size estimates, the following estimates for sample size were computed for the interaction effects:

1) *F* test on sR^2 for set 3; $f^2 = .29$; $n^* = 38$.

Thus, the present sample size of 45 is adequate to reject each null hypothesis for the interaction effects, given the effect sizes estimated above.

Statistical Analyses

Data were analyzed using SPSS statistics software (SPSS for Windows, Version 6.1.3, 1995). Univariate tests, t-tests and ANOVA for dichotomous and categorical variables (smoking history, family history of CAD, hypertension, NYHA functional angina class, number of diseased vessels) and zero-order correlations for continuous variables (age, weight) were used to examine risk factors and demographics. Variables that were significantly associated with coronary diameter changes were used in analyses as covariates.

Hypotheses examining the independent effects for severity of atherosclerosis, serum lipoprotein levels, and stress reactivity (Hypotheses 1, 2, 3, 5 and 6) were examined by way of zero-order correlations. For significant zero -order correlation, mean response differences in coronary responses were examined treating lipoprotein levels, severity of atherosclerosis and stress reactivity) as dichotomous variables based on median values. T-tests were used to examine mean difference in coronary response between these groups.

In order to examine the hypotheses concerning the interaction between stress reactivity, serum lipoprotein levels and severity of atherosclerosis (Hypotheses 4 and 7), a set-wise hierarchical multiple regression procedure was used (Cohen & Cohen, 1983). This procedure involves entering sets of variables (including sets with 1 independent variable) cumulatively in a specified order. The total proportion of variance (R^2) of the dependent variable (Y) is determined at each stage, indicating the additional variance due to each set (sr^2) having partialled the Y variance from sets higher in the model. Specifically, demographic and clinical variables significantly related to the dependent variable were entered first as a set of covariates. The independent predictors (i.e., severity of atherosclerosis, serum lipoprotein levels and stress reactivity) were entered in the second set. The 2-way interaction terms (severity of atherosclerosis X stress reactivity, serum lipoprotein levels X reactivity) were entered in the third set of each hierarchical model in order to test the additional variance predicted over the main effects (see also Cohen & Cohen, 1983, p. 301-350). In addition, the interaction of stress reactivity and atherosclerosis was examined using a repeated-measures ANOVA. This model entered hypertension as a covariate and examined stress reactivity (blood pressure, heart rate, and mood responses) as between-

subjects factors interacting with type of segment (lesion versus smooth) as a within-subjects factor. In order to visually represent the data from significant interactions, the regression equation is used to calculate y for the 4 cells of a 2 X 2 interaction. For the 2 independent variables of interest, each variable is entered into the regression equation at one standard deviation above or below the mean. This gives 4 conditions corresponding to 1) high and high, 2) high and low, 3) low and high, and 4) low and low, for the 2 independent variables. As opposed to values based on median splits of the independent variable, this procedure creates a numerical representation of the interaction that is based on the regression equation.

RESULTS

Results are presented in the following order: First, patient characteristics (i.e., demographics, clinical status) and mean hemodynamic and psychological responses to stress are presented, followed by an examination of the mean epicardial vasomotion in both atherosclerotic and smooth coronary segments for the study group as a whole. Next the principle hypotheses (i.e., effects of severity of atherosclerosis, lipoproteins, stress reactivity, and the interaction of these factors on epicardial vasomotion to mental stress) are addressed. While the hypotheses are divided according to segment type (atherosclerotic versus smooth), the results, for greater clarity, are presented according to the dependent variables. First, results are discussed for the independent effects of atherosclerotic severity (Hypothesis 1) and stress reactivity (Hypotheses 2,5) on vasomotion followed by the interactive effects of

atherosclerotic severity with stress reactivity on vasomotion (Hypothesis 4). Next, results are examined concerning the effects of serum lipoprotein levels (Hypotheses 3,6) and the interaction of stress reactivity and serum lipoprotein levels on vasomotion (Hypotheses 4,7). Presented last is a post-hoc analysis of the possible effects of patient gender on the results concerning smooth coronary segments. For summary purposes and ease of reference, a brief summary of data confirming and not confirming each hypothesis is outlined on page 74.

Patient Characteristics

Demographics. Of the 51 patients enrolled in the study, 6 patients were excluded prior to analysis due to technically inadequate angiographic film quality. The remaining 45 patients, 39 males and 6 females, had a mean age of 58.7 $\pm$ 10.3 years, ranging from 37 to 75 years of age. Mean years of education was 12.8 $\pm$ 3.5 ranging from 4 to 20 years. Thirty-three (73%) of the patients were Caucasian, 11 (24%) were African American, and 1 (2%) was Native American.

Clinical Status. Among the 45 patients, 41 (91%) had a recent prior positive exercise test or thallium test indicative of myocardial ischemia. Four patients (9%) had either a nondiagnostic test or unavailable data. Among the 45 patients, 5 (11%) had New York Heart Association Class I anginal symptoms, 12 (26%) Class II, 17 (37%) Class III and 2 (4%) Class IV. Data were unavailable on 9 (20%) patients. Initial cardiologist classification at the time of diagnostic catheterization was used to classify patients as 0, 1, 2, or 3 vessel disease. This variable was examined as a possible covariate. Twelve (27%) patients had

angiographically smooth coronary arteries, 14 (31%) had 1 vessel disease, 7 (16%) had 2 vessel disease, and 12 (27%) had 3 vessel disease. Of the 30 patients with available data the mean ejection fraction was 59.8% $\pm$ 15.2. Three patients evidenced left ventricular ejection fraction $\leq$ 30% at the time of catheterization.

The mean total serum cholesterol level for the sample was 209.5 $\pm$ 50.7 mg/deciliter. Mean LDL was 137.8 $\pm$ 50 mg/deciliter with a mean HDL of 39.5$\pm$ 15.5 mg/deciliter. Twenty-six (57%) patients were positive for hypertension (current hypertension or treated medically) and 18 (40%) were current cigarette smokers. Twenty-nine patients (64%) had a positive family history of CAD (24 with first degree relative and 4 with second degree relative).

All 45 patients underwent mental-stress testing. Nitroglycerine was not administered to 3 patients (7%). Although anti-ischemic medications were held prior to catheterization for all study patients when clinically possible, 4 patients (8%) remained on anti-ischemic medications during the catheterization. Three patients remained on a calcium-channel blocker and 1 patient remained on a beta-blocker. Of the 45 patients, 8 (17%) were administered pre-catheterization sedation. Neither administration of pre-catheterization sedation or maintenance of anti-ischemic medication was related to epicardial vasomotion in the present sample. LDL values were missing for 3 patients and HDL values were not obtained for 1 patient. Mean values were not substituted for missing values in order to maintain full sample size for calculations, instead these subjects were omitted for analyses involving LDL or HDL (and their interactions). Regression analyses used a pairwise procedure such that subjects with missing data points were not deleted from the entire model.

Hemodynamic and Psychological Responses To Mental Stress

Table 1 summarizes the hemodynamic and psychological responses to mental stress. Patients demonstrated a significant increase in SBP, DBP, mean arterial pressure (MAP), and HR to mental stress. Individual patients demonstrated a large range of responses (range MAP response = -3 mm/Hg to 42 mm/Hg; range HR response = -3 bpm to 37 bpm). MAP, which was used as the primary measure of blood pressure in order to limit the number of predictor variables in the analyses, was highly correlated with SBP, r=0.93, and DBP, r= 0.96. Repeated measures ANOVA revealed no significant differences for heart rate and mean arterial pressure among the sequential 30-second time periods for the mental stress task (all p's >.10). That is, the mental stress task induced a relatively immediate and stable increase across the task.

Anger, frustration, and irritation responses to mental stress were intercorrelated (r's between 0.51 and 0.76). To reduce the number of predictor variables, these 3 self-report measures were combined to form a composite Negative Affect variable. This composite variable correlated with anger, irritation and frustration (r's between 0.81 and 0.90). As seen in Table 1, there was a significant increase in Negative Affect during mental stress.

Epicardial Vasomotion In Atherosclerotic Segments and Smooth Segments

A total of 78 coronary segments (45 angiographically smooth segments and 33

atherosclerotic segments) in 45 patients were analyzed for the present study. Atherosclerotic segments were chosen at diagnostic catheterization and evidenced $\geq$ 25% stenosis at quantitative coronary angiography. The 33 atherosclerotic segments had a mean stenosis of 55% $\pm$ 15 ranging from 27%-86%. Epicardial vasomotion to mental stress and nitroglycerine is presented as percent change from baseline with negative values representing vasoconstriction and positive values representing dilation.

The mean diameter of the 33 atherosclerotic segments was 2.22 mm ($\pm$ 0.65) at baseline and 2.21 mm ($\pm$ 0.70) at mental stress. The vasomotor response ranged from 15% constriction to 17.7% dilation. There was no significant change in mean diameter (-0.40%$\pm$ 8.6) for the entire sample [$t(1,32)=0.27$, p=NS]. The 33 atherosclerotic segments dilated significantly to nitroglycerine with a mean dilation of 6.0% $\pm$ 12 ($t=2.72$, $p<0.01$). Education, age, smoking status, anginal class, family history of CAD, number of vessels diseased, and hypertension were not related to epicardial constriction or dilation in the 33 atherosclerotic segments.

The mean diameter of the 45 angiographically smooth segments at baseline was 2.91 mm $\pm$0.67 and 2.90 mm $\pm$0.65 at mental stress. The vasomotor responses ranged from 13.2% constriction to 12.4% dilation (mean change = -0.32%$\pm$ 5.5). In the 45 smooth segments, hypertension was the only demographic or risk factor significantly related to epicardial vasomotion ($t=2.65$; $p<.01$). Patients positive for hypertension tended to evidence epicardial constriction to mental stress (-1.8%$\pm$ 4.6) while patients without hypertension tended towards epicardial dilation (2.3%$\pm$ 5.6). Therefore, all analyses of smooth coronary segments entered hypertension as a covariate. The 45 smooth segments significantly dilated

to nitroglycerine (mean dilation: 5.6% ± 11 [t(1,44)=3.42, p<0.001].

Independent Effects of Severity of Atherosclerosis and Stress Reactivity on Epicardial Vasomotion

Hypothesis 1 predicted that severity of atherosclerosis would be related to greater vasoconstriction to mental stress. This hypothesis was tested in two ways. First, the hypothesis was examined among the atherosclerotic segments only, predicting that more severe atherosclerosis would relate to more severe constriction to mental stress. Zero-order correlations revealed no significant correlation between severity of atherosclerosis and diameter change to mental stress (see Table 2), indicating that among diseased vessels, a greater degree of atherosclerosis was not related to greater coronary constriction. A second test of Hypothesis 1 compared coronary responses to mental stress between atherosclerotic segments and angiographically smooth segments. Specifically, a paired t-test comparing the response of 33 atherosclerotic segments to the 33 smooth segments, in the same patients, revealed no significant difference in epicardial diameter response to mental stress (mean vasomotion: -0.2%± 5.5 for smooth segments; -0.4%±8.6 for lesion segments).

Hypotheses 2 and 5 predicted that greater stress reactivity (blood pressure, heart rate, and negative affect responses) would predict coronary constriction in atherosclerotic segments and predict greater dilation in angiographically smooth segments, respectively. Looking at atherosclerotic segments first, zero-order correlations revealed that blood pressure response to stress was significantly negatively related to vasomotion, with higher MAP

response to mental stress relating to greater constriction ($r=-0.43$, $p<.01$; see Table 2, see Figure 2 for scatterplot). In addition, this hypothesis was tested by categorizing patients as high or low MAP reactors to mental stress based on a median MAP response of 11.22 mm/Hg. T-tests examining blood pressure change as a dichotomous variable revealed a significant effect [$t(1,32)=2.55$; $p<.02$] with high blood pressure reactors tending to constrict to mental stress (mean change = -3.8%$\pm$ 7.1) and low blood pressure reactors tending to dilate (mean change = 3.2% $\pm$ 8.7). No independent relationship was found for heart rate or negative affect responses and coronary vasomotion in atherosclerotic segments.

While blood pressure was significantly related to coronary constriction in atherosclerotic segments, looking at the 45 smooth segments, no relationship was found between stress reactivity (blood pressure, heart rate, Negative Affect responses) and vasomotion (see Table 2).

A significant stress reactivity effect in atherosclerotic segments and no significant effect in smooth segments (presented earlier) suggested a possible interaction of stress reactivity and atherosclerosis (Hypothesis 4). Two types of analyses are presented below to directly test Hypothesis 4. First, the effect of stress reactivity on coronary vasomotion is compared between 33 atherosclerotic segments and 33 smooth segments in the same patients. Second, the effect of stress reactivity on coronary vasomotion is examined among the 33 atherosclerotic segments in order to determine the interactive effects of stress reactivity with the degree of atherosclerotic severity.

Interaction of Stress Reactivity and Atherosclerosis in Predicting Epicardial

Vasomotion

A repeated-measures approach comparing the 33 atherosclerotic segments and the 33 smooth segments from the same patients was used to address Hypothesis 4. This analysis examines whether the relationship of stress reactivity to epicardial vasomotion differs in atherosclerotic as compared to smooth coronary segments. Patients were categorized as high or low MAP reactors to mental stress based on a median MAP response of 11.22 mm/Hg. Results revealed a main effect for MAP response ($F(1,16)=9.60$, $p<.005$) which was qualified by a significant MAP response by segment type interaction ($F(1,27)=5.57$, $p<.03$). As predicted, means indicate that in atherosclerotic segments high MAP reactors tended to constrict (mean change = -3.8% $\pm$ 7.1) whereas low MAP reactors tended to dilate (mean change = 4.0% $\pm$ 8.4)(see Figure 3). However, vasomotion in smooth coronary segments did not differ between high and low blood pressure reactors (see Figure 3).

Patients were categorized as high or low HR reactors to mental stress based on a median HR response of 9.60 bpm and results also revealed a significant HR response by segment type interaction ($F(1,27)=9.60$, $p<.005$). As predicted, means indicate that atherosclerotic segments in high HR reactors tended to constrict (mean change = -2.6% $\pm$ 8.5) while smooth coronary segments in high HR reactors tended to dilate (mean change = 1.7% $\pm$ 4.5)(see Figure 4). Means also revealed that atherosclerotic segments in low HR reactors tended to dilate (mean change = 2.3% $\pm$8.5) whereas smooth segments in low HR reactors tended to constrict (mean change = -2.5% $\pm$ 6.2)(see Figure 4). No interaction effect was revealed for Negative Affect responses by segment type (atherosclerotic compared to

smooth).

Hypothesis 4 states that stress reactivity would interact with severity of atherosclerosis in predicting epicardial vasomotion and was also examined within the 33 atherosclerotic segments. Regression analyses revealed no significant interactions for blood pressure, heart rate, or negative affect responses with severity of atherosclerosis among atherosclerotic segments.

Independent Effects of Lipoproteins on Epicardial Vasomotion

Hypotheses 3 and 6 predicted that serum lipoproteins would be related to vasomotion in both atherosclerotic and smooth epicardial segments, respectively. Zero-order correlations among atherosclerotic segments revealed an unexpected, but marginally significant, negative correlation between HDL and vasomotion with higher levels of HDL being related to greater constriction (see Table 3). There was no significant relationship between LDL and vasomotion among atherosclerotic segments. Zero-order correlations among smooth coronary segments revealed no significant relationship for serum lipoprotein levels (see Table 3).

Interaction of Stress Reactivity and Lipoproteins in Predicting Epicardial Vasomotion

Hypotheses 4 and 7 predicted that stress reactivity would interact with serum lipoprotein levels in predicting epicardial vasomotion in both atherosclerotic and smooth

coronary segments. Among the atherosclerotic segments, multiple regression analyses revealed no significant interactions for blood pressure, heart rate, or negative affect responses with either LDL or HDL levels.

However, among the smooth coronary segments multiple regression analyses revealed a significant interaction effect for MAP by LDL (see Table 4, regression results). The overall model was significant [F, r^2 change (4,35)=2.79, $p<.04$]. The LDL by MAP response interaction added significantly to hypertension, MAP response, and LDL in predicting vasomotion (F (4,35)=4.12, $p<.05$). Vasomotion responses to mental stress for high and low MAP reactors by high and low LDL are presented in Table 5. The direction of the mean responses among the groups are not as predicted. Among the high MAP reactors, low LDL patients tended to dilate while the high LDL patients tended to constrict. Among the low MAP reactors, the high LDL patients displayed the most constriction.

Multiple regression analyses also revealed a significant interaction effect for LDL by heart rate (see Table 6). The overall regression model in this analysis was significant [F(4,35)=3.64, $p<.01$]. The LDL by heart rate response interaction added significantly to hypertension, LDL, and heart rate response [F, r^2 change (4,35)=6.50, $p<.01$]. Vasomotion to mental stress for high and low heart rate reactors by high and low LDL are presented in Table 7. Again the direction of the interaction is not as predicted. Among high heart rate reactors, low LDL was related to dilation. However, among low heart rate reactors, low LDL was related to constriction.

Possible Effects of Gender on Results in Smooth Coronary Segments

In the present sample, female patients were more likely to have smooth coronary arteries (5 of 6 females were free of coronary disease). In addition, female patients had significantly different blood pressure reactivity to stress, LDL levels, and marginally different ($p<.09$) HDL levels than the male patients (see Table 8). Two of the independent predictors (stress reactivity and serum lipoprotein levels) differed significantly between male and female patients and almost all female patients were completely free of coronary disease. Because of these comprehensive gender differences we sought to assess whether the inclusion of women in the analyses systematically altered the results. Specifically, a reanalysis of coronary vasomotion was conducted with male patients only to assess whether the predicted relationships were more evident among the males.

Reanalysis among male patients only revealed that hypertension remained significantly related to smooth segment vasomotion [$t(1,38)=2.15$, $p<.04$] with non-hypertensive patients tending to dilate to mental stress (2.2%$\pm$ 5.8) and hypertensive patients tending to constrict (-1.6%$\pm$ 4.9). Among the male sample, LDL was not significant as a predictor of epicardial vasomotion in smooth segments among male patients ($r = -0.25$, $p<.14$). However, as seen in Figure 5, 2 patients were statistical outliers (LDL < 60). The correlation coefficient presented in Figure 5 represents the relation of LDL to epicardial vasomotion without the 2 outliers ($r = -0.48$, $p<05$). These data reveal that higher levels of LDL, particularly greater than 190, are related to constriction to mental stress while low levels of LDL are related to coronary dilation.

Analysis of the interaction between stress reactivity and serum lipoprotein levels among men (Hypothesis 7) revealed that LDL by MAP response interaction remained

significant (see Table 9 for regression analysis). The overall regression model for this analysis was significant $F(4,30)=8.06$, $p<.0002$). The MAP x LDL interaction term added significantly to hypertension, LDL, and MAP response [F, r^2 change $(4,30)=21.6$, $p<.0001$]. To display the nature of this effect, male patients were categorized as high and low MAP reactors and high and low LDL based on the regression equation. Vasomotion responses to mental stress indicate that among low blood pressure reactors, there was no effect of LDL on vasomotion (see Table 10). However, among high blood pressure reactors, low LDL was associated with dilation and high LDL was associated with constriction.

The LDL by HR response interaction among men was marginally significant (see Table 11 for regression analysis). The HR x LDL interaction term added marginally to hypertension, LDL, and HR response [F, r^2 change $(4,30)=3.28$, $p<.08$; overall model $F(4,30)=2.41$, $p<.07$). Vasomotion to mental stress for HR reactors and LDL levels are presented in Table 12. Values indicate that among low HR reactors there was no effect of LDL. However, among high HR reactors, patients with low LDL tended to dilate whereas patients with high LDL did not tend towards dilation or constriction.

In contrast to the entire sample, the HDL by MAP response interaction was significant among male patients (see Table 13 for regression analysis). The overall regression model was significant for this analysis $[F(4,32)=3.03, p<.03]$ among male patients. The HDL x MAP interaction term added significantly to hypertension, HDL, and MAP response [F, r^2 change$(4,32)=6.25$, $p<.02$). Vasomotion to mental stress for HDL and MAP reactivity are presented in Table 14. Despite the significant interaction and the large r^2, the mean values in each cell are close to 0.0 and the nature of the interaction is difficult

to assess.

Summary of Hypotheses

Hypotheses for Atherosclerotic Segments:

1. Severity of atherosclerosis: Not confirmed (within atherosclerotic segments (n=33) or between atherosclerotic and smooth segments (n=66)).
2. Magnitude of stress reactivity: Confirmed (MAP related to coronary vasomotion).
3. Serum lipoproteins: Not confirmed.
4 Interaction atherosclerosis and reactivity: Confirmed (Reactivity related to vasomotion in atherosclerotic but not smooth segments; Not confirmed within atherosclerotic segments).

   Interaction of serum lipoproteins and reactivity: Not confirmed.

Hypotheses for Angiographically Smooth Coronary Segments:

5. Magnitude of reactivity: Not confirmed.
6. Serum Lipoproteins: Not confirmed in entire sample (see preliminary evidence in male subjects regarding LDL).
7. Interaction of serum lipoproteins and reactivity: Confirmed (see direction of effects for limitations in confirmation).

DISCUSSION

The present research examined the moderators of the effects of mental stress on epicardial coronary artery constriction and dilation. Specifically, the effect of severity of atherosclerosis, serum lipoprotein levels, and stress reactivity (blood pressure, heart rate, and negative affect responses) were examined as predictors of epicardial vasomotion to mental stress in both atherosclerotic and smooth coronary segments. In addition, the relationship of stress reactivity and coronary vasomotion was hypothesized to differ depending on atherosclerotic severity and serum lipoprotein levels.

The set of hypotheses in this study are derived from the premise that the functional status of the epicardial endothelium varies with atherosclerosis and differing serum lipoprotein levels. A greater degree of atherosclerosis, higher levels of LDL, and lower levels of HDL would be related to relatively greater endothelial dysfunction. We predicted that stress reactivity would then, in turn, interact with these factors. Greater reactions to stress should elicit a tendency to constrict given relatively greater endothelial dysfunction. However, greater reactions to stress should elicit either less constriction or perhaps dilation given relatively better preserved endothelial function.

Effects of Atherosclerotic Severity and Stress Reactivity on Coronary Vasomotion

An underlying hypothesis of the present study was that mental stress may elicit constriction in atherosclerotic segments, but elicit either dilation or have no effect in smooth coronary segments. This hypothesis is based on the premise that atherosclerotic segments have greater endothelial dysfunction than smooth coronary segments. Contrary to this

reasoning, the present findings reveal no overall effect of constriction or dilation to mental stress in atherosclerotic or smooth segments. These results, instead, provide evidence that there are specific moderators of the response to mental stress. Specifically, results in atherosclerotic segments revealed that the magnitude of physiological reactivity to stress moderated the vasomotion response. However in smooth coronary segments, hypertension, the interaction of high stress reactivity and lipoprotein levels (LDL) and possibly the independent effect of LDL moderate vasomotion.

The present results did not support hypothesis 1 or 4, that, among a sample of atherosclerotic segments, greater severity of atherosclerosis would be associated with greater epicardial constriction, particularly among high reactors to stress. Among lesions ranging from 27% stenosis to greater than 80% stenosis, greater severity of stenosis did not relate to constriction, nor did greater severity of atherosclerosis and high reactivity relate to greater coronary constriction. One possible reason for this finding is that above a certain threshold of disease (e.g., visible lesion) greater severity of atherosclerosis does not appear to influence endothelial dysfunction. However, evidence from prior research that indicates "stenosed" segments (generally defined as greater than 40% or 50% stenosis) may constrict more than "irregular" segments (defined as minimal disease up to 40% stenosis (Yeung et al., 1991; Nabel et al., 1990; Nabel et al., 1988; Ludmer et al., 1986). The present study, using quantitative coronary angiography as a more precise measure of lesion severity, does not support the hypothesis that endothelial dysfunction worsens dramatically as lesions severity progresses from minimal lesions (30%) to more severe (50-80%).

Previous findings are inconsistent with respect to the response of arterial segments to mental stress. Some studies demonstrate an overall effect of mental stress in coronary

segments, with severely diseased, moderately disease, or smooth coronary segments evidencing constriction to mental stress (Yeung et al., 1991; Dakak et al., 1995; Lacey et al., 1995). Other mental stress studies reveal no overall constriction or dilation to mental stress (Boltwood et al., 1993; Labatte et al., 1991).

In atherosclerotic segments, Hypothesis 2 predicted that stress reactivity would be related to vasomotion such that greater reactivity would be related to greater epicardial constriction. This hypothesis received support for blood pressure responses, with higher MAP responses to mental stress associated with greater constriction in atherosclerotic segments. It is possible that the effect of reactivity on coronary vasomotion reflects the fact that mental stress, as compared to other stimuli such as acetylcholine, is a variable stimulus (Krantz & Manuck, 1984) with physiological reactivity ranging from none to somewhat large response across patients. Therefore it is possible that the above-mentioned lack of an overall constrictive effect of mental stress in atherosclerotic segments may be due to the fact that a subset of the patients in studies reporting no differences may not react to the stress task enough for it to be a viable stimulus. If this were true, an overall effect may not be revealed in atherosclerotic segments or may be revealed only in the higher reactors. This is supported in the present data. Figure 3 revealed that among high blood pressure reactors, atherosclerotic segments constricted approximately 4%, and smooth segments did not dilate or constrict. However these percent changes are still relatively small compared to those obtained during other mental stress studies (9% and 12% constriction in moderately diseased segments and 24% constriction in stenosed segments, see Yeung et al ., 1991; Dakak et al., 1995).

Additional support would be found for the hypothesis that variable stress responses

may cause the lack of an overall effect for mental stress in the present study, if those studies that revealed an overall constriction effect for atherosclerotic segments induced higher mean reactivity to stress or less variable responses among the patients. However there were no apparent differences in the magnitude of stress reactivity between the present study and the mental stress study eliciting the greatest constriction (Yeung et al., 1991).

Although the present results reveal that the magnitude of stress reactivity is a moderator of vasomotion in atherosclerotic segments, part of the correlation effect obtained is due to the fact that, among atherosclerotic segments, low blood pressure reactors dilated to mental stress (see Figure 3). Among atherosclerotic segments where endothelial function is presumably poor, high reactivity is predicted to elicit constriction but there is no clear explanation for the dilation of atherosclerotic segments among the low reactors in the present study. While some explanations may be plausible (e.g., the low reactors had less endothelial dysfunction due to less lower reactivity over time) explanations of this type cannot explain the fact that these same subjects did not show dilation of the smooth segments (see Figure 3). It is clear that systemic or trait-like explanations for the dilation in the atherosclerotic segment should also affect the smooth segments; therefore the data suggest that atherosclerotic versus smooth segments may be functionally different with respect to factors mediating coronary vasomotion. That is, only a difference in some local process between the segment types can explain this phenomenon. The fact that the magnitude of reactivity is a moderator of the vasomotor responses to mental stress in atherosclerotic segments may reflect that there is already enough endothelial dysfunction so that high reactivity in itself can elicit coronary constriction.

The results of this study indicate that the moderators of vasomotion differ in smooth

stenosed coronary segments. Such an interaction between type of segment and stress reactivity is suggested by the fact that higher blood pressure responses were related to greater coronary constriction in atherosclerotic segments, but there was no effect of stress reactivity among the smooth coronary segments (disconfirming Hypothesis 5). Moreover, when the interaction was directly examined by comparing atherosclerotic and smooth segments from the same subjects in the same analysis, a significant interaction effect for stress reactivity and lesion severity (i.e., atherosclerotic segments versus non-atherosclerotic segments) was evident for both blood pressure and heart rate responses. This finding indicates that blood pressure reactivity was related to epicardial diameter changes in atherosclerotic segments but not in smooth coronary segments. While the significant interaction for heart rate responses appears to provide further support that the moderators of vasomotion are different between smooth and atherosclerotic segments, an examination of the mean changes in vasomotion reveals a more complex pattern. Among high heart rate reactors, there was, as predicted, a tendency for atherosclerotic segments to constrict and smooth segments to dilate. However, among low heart rate reactors, where little effect was expected, atherosclerotic segments tended to dilate and smooth segments tended to constrict. The effect of type of segment (smooth, atherosclerotic) within the low HR reactors group contributes to the significance of the interaction and there were no effects for HR on vasomotion revealed when smooth and atherosclerotic segments were analyzed separately. Therefore, it is plausible to suggest that blood pressure responses are a more reliable predictor of coronary vasomotion in the present sample than HR responses.

Moderators of Vasomotion in Smooth Coronary Segments

The interaction results described above provide further evidence that stress reactivity, while a moderator of vasomotion in atherosclerotic segments, is not, on its own, a moderator of vasomotion in smooth coronary segments. Instead, the present results indicate that coronary vasomotion in smooth coronary segments is moderated by the joint effects of stress reactivity and LDL. Both blood pressure and HR responses interacted with LDL levels to predict vasomotion in smooth coronary segments. While the direction of the interaction is slightly closer to predictions among the male sample, the mean values for the change in coronary diameter revealed minimal support for the hypotheses (see Tables 5 and 7).

The present results suggest that in smooth coronary segments, moderators such as LDL are important together with stress reactivity in predicting coronary vasomotion. Results also suggest that LDL alone and hypertension, in males, may play a moderating role on the vasomotion response to mental stress (see below). Prior research with mental stress has revealed that smooth segments may show either a tendency towards dilation or an overall constriction (Yeung et al, 1991; Lacey et al., 1995). These apparently discrepant results from smooth coronary segments likely reflects a wide range of underlying endothelial disfunction within smooth coronary arteries. As stated, the present results indicate that the presence of factors such as low density lipoprotein levels that may affect endothelial function to a greater degree in smooth coronary arteries. Although this is the first study using mental stress to demonstrate such an effect, evidence from acetylcholine studies and cold pressor studies reveal that factors such as lipoproteins and coronary risk factors are most likely to play a moderating role in smooth coronary segments (e.g., Vita et al, 1990; Zeiher et al., 1991). Because coronary endothelial function may vary widely among smooth segments, an increase in reactivity to stress may cause dilation in some patients and constriction in others,

such that stress reactivity is not an independent moderator. However, stress reactivity in conjunction with other variables affecting endothelial function (e.g., LDL) may moderate the response to mental stress in smooth coronary segments.

Effects of Lipoproteins and Coronary Risk Factors on Coronary Vasomotion

In the present study lipoprotein levels and coronary risk factors appeared to be more useful in predicting vasomotion in smooth segments (preliminary evidence for Hypothesis 6) as compared with atherosclerotic segments (not supporting Hypothesis 3). The only coronary risk factor or demographic factor significantly related to vasomotion was hypertension, and this was found only in smooth coronary segments. In addition, stress reactivity interacted with lipoprotein levels in smooth segments and not in atherosclerotic segments. Reanalysis of the smooth segments among male patients only, uncovered preliminary evidence of an independent effect for LDL, with greater levels of LDL relating to greater constriction. The only finding relating lipoprotein levels or risk factors to coronary vasomotion in atherosclerotic segments was a marginal effect for HDL, and this was in the opposite direction of that predicted. As discussed above, prior research supports this finding in that studies using stimuli other than mental stress, such as cold pressor or acetylcholine, generally report significant effects for coronary risk factors or for lipoproteins in smooth coronary segments (e.g., Vita et al., 1990; Kuhn, et al 1990). One possible reason for this finding is that in atherosclerotic segments the progress of atherosclerotic disease (and endothelial dysfunction) is so advanced that other variables may be less likely to play large role. In smooth segments, where endothelial damage is in its initial stages, LDL or other

coronary risk factors (e.g., hypertension in the present study) may be more important in determining the overall level of endothelial function.

Psychological Responses to Mental Stress and Coronary Vasomotion

The present findings of a lack of an effect of psychological responses to stress in predicting vasomotion in either smooth or atherosclerotic segments is in contrast to the findings of Boltwood et al. (1993). However, the findings of Boltwood et al. were based on only 7 patients, and it is therefore possible that the significant results were due to chance. It should be noted that prior research has demonstrated that self-reported psychological responses to stress are often not highly related to physiological responses (Krantz & Manuck, 1984). However, because Boltwood et al.(1993) used a 10-question state anger scale and the present study used a different measure of anger and negative affect, the present study can not be viewed as a failed replication.

Effect of Gender on Stress-induced Vasomotion in Smooth Coronary Segments

In the present sample, female patients evidenced significantly lower physiological stress reactivity, significantly lower LDL levels, and marginally higher HDL levels than male patients. Therefore, when combined with the fact that 5 of 6 of the female patients had either endogenous or exogenous estrogen present, it raised the possibility that males and females were distinct with respect to the current hypotheses. When analyses were repeated with males only, the interaction terms remained significant and the directions were slightly closer

to those predicted. In addition, a possible LDL effect emerged with greater LDL levels associated with relatively greater constriction. One possibility for these results is that the predicted relationships do not hold in this particular sample of women. That is, by chance the data were cleaner when omitting the female patients in the present sample. However, it is also possible that the predicted relationships do not hold for females in the general population and that future studies should specifically examine gender when addressing these research questions. It is also possible that other moderators that were not examined in the present study, such as estrogen levels, may play a more crucial role in moderating coronary vasomotion in female patients (see Reis et al., 1994).

Study Limitations

One limitation of the present study is the underrepresentation of females subjects. The study must be viewed as a study of male CAD patients with conclusions not applying to female patients. As indicated above, the present results seem to indicate that female patients do in fact differ with respect to stress reactivity and lipoprotein levels and that future studies should specifically address the possibility that predictors of vasomotion may differ between female and male subjects.

One possible limitation of the present study is the relatively small mean changes in coronary diameter size obtained to mental stress. While the diameter changes in the present study are similar to those seen in Lacey et al. (1995)(approximately 6% constriction), they are less than changes seen in Yeung et al. (1991)(24% and 9 % constriction in severe and moderate stenosis) and Dakak et al. (1995)(12% constriction). It is possible that the changes

obtained in the present study are small enough such that they are of little clinical relevance and therefore the findings merely contribute to our understanding of the general physiological and biochemical basis of coronary vasomotion to mental stress. That is, the changes in coronary diameter in the present study may reflect underlying disease processes (endothelial dysfunction), but still not necessarily cause a large enough change in epicardial diameter to be clinically significant. Indeed, some researchers have noted that it is possible that changes in the microvasculature may be more crucial to coronary blood flow than the relatively small changes seen in the epicardial arteries (Yeung et al.,1991; Dakak et al., 1995).

However, it is also possible that relatively small changes in epicardial constriction may be important clinically. First, as proposed by Muller et al., (1994) it is possible that constrictive forces in the epicardial arteries may relate to plaque rupture and consequent myocardial infarction. In addition, relatively small changes in epicardial diameter may play a role in myocardial ischemia given that most ischemia is thought to be due to a combination of factors including fixed lesions, platelet aggregation, vasoconstriction of the epicardial vessels, reduced dilation of the resistance vessels, and increases in myocardial demand (Cohn, 1992; Braunwald & Sobel, 1992; Rutherford & Braunwald, 1992). In the present sample, a patient with high stress reactivity and high levels of LDL tended to evidence constriction in both atherosclerotic and smooth epicardial arteries. Since epicardial responses are seen as a marker for endothelial dysfunction, it is also probable that, in these patients the microvasculature is not dilating as it should (see Dakak et al., 1995). When combined with the greater demand on the heart from the increases in contractility and heart rate it becomes evident that a relatively small epicardial diameter change may be part of a

larger process.

Another possible limitation of the present study is the use of mean diameter change across the length of the lesion as opposed to percent change at the point of minimal luminal diameter. Changes in minimal luminal diameter may be more clinically significant when examining ischemia due to the fact that flow is influenced more by the narrowest point in the arterial segment. However, because the minimal luminal diameter is based on one point as opposed to a mean diameter change across the length of the lesion, it is less reliable. This may be particularly true given the possible error inherent in the data due to problems with angiographic film quality (see below). While the use of minimal luminal diameter may be preferable for certain reasons, the use of the mean diameter change across the lesion length should not affect comparison with other mental stress studies. Of mental stress studies examining atherosclerotic segments, Yeung et al. (1991) used mean diameter changes, two studies examined both minimal luminal diameter changes and mean diameter changes (Boltwood et al., 1993; Labatte et al., 1992), while Dakak et al. (1995) examined minimal luminal changes only. The two studies examining both measures revealed no difference in results. However, it remains unclear, given the use of a reliable measure of minimal luminal diameter, whether the same moderators would help predict vasomotion changes in the present sample.

Another limitation of this present study is the fact that direct testing of coronary segments for endothelial function was not conducted. While acetylcholine administration per se does not assess responses to real-life triggers of coronary vasomotion, it provides a consistent pharmacological stimulus to the endothelium across all patients and allows for a more direct assessment of endothelial function than mental stress. The hypotheses in the

present proposal and interpretation of the results are based on the idea that certain moderators may affect endothelial function and that stress reactivity will then interact with endothelial function. Vasomotion data from acetylcholine infusion would have allowed a direct test of these underlying assumptions made in the present study. Future research using acetylcholine and mental stress testing could assess whether the moderator variables in the present study (e.g., stress reactivity, LDL) help predict coronary responses to mental stress while controlling for the effect of acetylcholine. That is, researchers could begin to examine what variables are important in predicting coronary responses to complex stimuli, such as mental stress, over and above the predictive value of an objective measure of endothelial dysfunction (i.e., coronary responses to acetylcholine). Based on the results of Yeung et al (1991) approximately 65% of the variability in the coronary response to mental stress is not explained by the coronary response to acetylcholine. By examining the coronary responses to both mental stress and acetylcholine in the same patients, researchers may begin to understand what factors, in addition to endothelial dysfunction, may have clinical relevance for the treatment and management of coronary artery disease.

Variations in the quality of the angiographic films obtained at the study sites also posed a difficulty for this study. As noted in the methods, 6 patients were excluded prior to analyses due technically inadequate film quality. In addition, reviews from the angiographic core laboratory indicated that some other films were usable, but were below average in image quality. It remains undetermined how, or whether, this variability in quality may have affected the results. It is possible that it merely introduced added error to the dependent measure, generally raising the variance in measured coronary diameter. In this regard, the standard deviation of the coronary responses in the present study were similar to those of

Dakak et al. (1995), but greater than Yeung et al. (1991) and Lacey et al. (1995), thus allowing for the possibility for increased error in the present sample. It is also possible that the poor angiographic film quality may have caused the relatively small diameter changes seen in the present study. If the relatively small diameter response was restricted to the mental stress task, it would be more likely that the task itself was the cause of the small responses. However, the coronary responses to nitroglycerine infusion also appear smaller [e.g., mean dilation in present study of 6% whereas the average dilation was 23% in Yeung et al. (1991)], thus allowing for the possibility that error could play a role in the overall response magnitude.

Summary

In the present study, we did not observe 1) an overall effect for mental stress to elicit constriction in atherosclerotic segments as compared to smooth coronary segments, or 2) that, on average, more severe lesions revealed greater constriction than less severe lesions. Instead, the present results provide evidence that the there are specific moderators of the epicardial responses to mental stress in both atherosclerotic and smooth coronary arteries. In atherosclerotic coronary segments, coronary vasomotion is moderated by the magnitude of stress reactivity with greater physiological reactivity being associated with greater coronary constriction. In accounting for this finding, it is theorized that endothelial dysfunction is far enough advanced in segments with atherosclerosis that the vasoconstrictive stimuli associated with stress reactivity (e.g., norepinephrine) can elicit coronary constriction. This is due to the fact that, because the endothelial dysfunction is greater, there

is less of an EDRF mediated dilation effect to counteract these constrictive forces.

The present results revealed that low density lipoproteins and coronary risk factors (hypertension), along with stress reactivity, are more predictive of coronary vasomotion in smooth segments than stress reactivity alone. In the case of smooth coronary segments, where endothelial function is presumably less advanced, coronary risk factors such as LDL or hypertension, join with stress reactivity in predicting coronary vasomotion.

Results also indicated that for the smooth coronary segments, the predicted relationships and the moderating relationships found in the present study may apply only to male patients. Future studies should address these gender differences directly by assessing a larger number of female coronary artery disease patients.

TABLES AND FIGURES

Table 1. Hemodynamic and Psychological Responses to Mental Stress. SBP = systolic blood pressure, DBP = diastolic blood pressure, HR = heart rate, MAP = mean arterial pressure, Negative Affect = a composite score of anger, irritation, and tenseness (see text for explanation). * $p<0.0001$, significant change from baseline.

	Baseline	Mental Stress	Change
SBP (mm Hg)	143.2 ± 23	157.1 ± 24	13.9 ± 14*
DBP (mm Hg)	75.1 ± 11	85.6 ± 14	10.5 ± 9*
HR (beats/min)	69.5 ± 10	81.1 ± 12	11.6 ± 9*
MAP (mm Hg)	97.8 ± 13	109.4 ± 16	11.6 ± 10*
Negative Affect	1.7± 1.1	3.9 ± 1.9	2.2 ± 1.9*

Table 2. Zero-order correlations between percent stenosis, stress reactivity (mean arterial blood pressure, heart rate, and Negative Affect) and percent change from baseline to mental stress in both atherosclerotic and smooth coronary segments. Values presented are Pearson correlation coefficients. MAP = mean arterial pressure, HR = heart rate.

	MAP Change	HR Change	Negative Affect Change	% Stenosis
Diameter Change in Atherosclerotic Segments (N=33)	-0.43 (p<.01)	-0.12(p=NS)	0.05(p=NS)	-0.10(p=NS)
Diameter Change in Smooth Segments (N= 45)	0.05(p=NS)	0.18(p=NS)	-0.05(p=NS)	

Table 3. Zero-order correlations between serum lipoprotein levels and percent change in epicardial diameter from baseline to mental stress. (Note: Significance is for individual correlation not for comparisons among correlations). Values presented are Pearson correlation coefficients.

	LDL	HDL
Percent Change in Atherosclerotic Segments (N=33)	-0.10(p=NS)	-0.32(p=.07)
Percent Change in Smooth Segments (N=45)	-0.10 (p=NS)	-0.02(p=NS)

Table 4. Hypertension, mean arterial pressure response to mental stress, serum LDL levels, and mean arterial pressure response by LDL interaction predicting epicardial vasomotion to mental stress in smooth coronary segments (*p<.05; "Δ in r^2 = change in r^2 due to addition of set).

Entry Order	Predictors (df)	F for Δ in r^2	r^2
1.	Covariate (1,38)	6.49*	.15
	Hypertension		
2.	Main Effect (3,36)	0.14	.15
	MAP change	-0.35(t)	
	LDL	-0.38(t)	
3.	Two way		
	Interaction (4,35)	4.12*	.24

Table 5: Predicted change in smooth segment diameter to mental stress: Interaction of serum LDL and mean arterial pressure response (Δ) to mental stress. Values are computed from the regression equation with "Low" and "High" corresponding to one SD below and above the mean, respectively (see text for explanation of procedure). "+" indicates dilation and "-" indicates constriction.

	LDL (Low)	LDL (High)
MAP change (Low)	+1.75%	-1.76%
MAP change (High)	-3.28%	+2.55%

Table 6. Hypertension, heart rate response to mental stress, serum LDL levels, and heart rate response by LDL interaction predicting epicardial vasomotion to mental stress in smooth coronary segments (*p<.05; Δ in r^2 = change in r^2 due to addition of set).

Entry order	Predictors (df)	F for Δ in r^2	r^2
1.	Covariate (1,38) Hypertension	6.49*	.15
2.	Main Effect (3,36) HR change LDL	0.35 0.73(t) -0.58(t)	.16
3.	Two way Interaction (4,35)	6.50*	.29

Table 7. Change in smooth segment diameter to mental stress: Interaction of serum LDL and heart rate response to mental stress. Values are computed from the regression equation with "Low" and "High" corresponding to one SD below and above the mean, respectively (see text for explanation of procedure). "+" indicates dilation and "-" indicates constriction.

	LDL (Low)	LDL (High)
HR change (Low)	-2.45%	+0.80%
HR change (High)	+3.28%	-0.76%

Table 8. Gender differences in stress reactivity and serum lipoprotein levels . MAP Δ = mean arterial pressure response to mental stress, HR Δ = heart rate change to mental stress, Negative Affect Δ = composite anger, irritation, frustration response to mental stress (* = $p<.05$; ** = $p<.005$).

	LDL**	HDL	MAPΔ*	HRΔ	Negative AffectΔ
Male (n=39)	146 (48)	36 (11)	12.8 (9.6)	12.1 (9.4)	2.1 (1.9)
Female (n=6)	85 (24)	58 (25)	4.0 (9.6)	7.7 (6.4)	3.2 (1.6)

Table 9. Hypertension, mean arterial pressure response to mental stress, serum LDL levels, and mean arterial pressure response by LDL interaction predicting epicardial vasomotion to mental stress in smooth coronary segments; male patients only (*p<.05; Δ in r^2 = change in r^2 due to addition of set).

Entry order	Predictors (df)	F for Δ in r^2	r^2
1.	Covariate (1,33)	4.22*	.11
	Hypertension		
2.	Main Effect (3,31)	1.07	.17
	MAP change	-0.81(t)	
	LDL	-1.29(t)	
3.	Two way		
	Interaction	21.59*	.52

Table 10. Change in smooth segment diameter to mental stress in male subjects only: Interaction of serum LDL and mean arterial pressure (MAP) response to mental stress. Values are computed from the regression equation with "Low" and "High" corresponding to one SD below and above the mean, respectively (see text for explanation of procedure). "+" indicates dilation and "-" indicates constriction.

	LDL (Low)	LDL (High)
MAP change (Low)	-0.09%	-0.10%
MAP change (High)	+2.1%	-1.11%

Table 11. Hypertension, heart rate (HR) response to mental stress, serum LDL levels, and heart rate response by LDL interaction predicting epicardial vasomotion to mental stress in smooth coronary segments; male patients only (*p<.05; Δ in r^2 = change in r^2 due to addition of set).

Entry order	Predictors (df)	F for Δ in r^2	r^2
1.	Covariate (1,33)	4.22*	.11
	Hypertension		
2.	Main Effect (3,31)	0.16	
	HR change	0.53(t)	
	LDL	-1.29(t)	
3.	Two way		
	Interaction (4,30)	3.27	.24

Table 12. Change in smooth segment diameter to mental stress in male subjects only: Interaction of serum LDL and heart rate response to mental stress. Values are computed from the regression equation with "Low" and "High" corresponding to one SD below and above the mean, respectively (see text for explanation of procedure). "+" indicates dilation and "-" indicates constriction.

	LDL (Low)	LDL (High)
HR change (Low)	-1.18%	-1.16%
HR change (High)	4.6%	0.02%

Table 13. Hypertension, mean arterial pressure (MAP) response to mental stress, serum LDL levels, and mean arterial pressure response by LDL interaction predicting epicardial vasomotion to mental stress in smooth coronary segments; male subjects only (*p<.05; Δ in r^2 = change in r^2 due to addition of set).

Entry order	Predictors (df)	F for Δ in r^2	r^2
1.	Covariate (1,35)	4.47*	.11
	Hypertension	-3.71	
		1.75	
2.	Main Effect (3,33)	0.37	.13
	MAP change	-0.53(t)	
	HDL	-0.68(t)	
3.	Two way Interaction (4,32)	6.25*	.27

Table 14. Change in smooth segment diameter to mental stress in male subjects only: Interaction of serum HDL and mean arterial pressure response to mental stress. Values are computed from the regression equation with "Low" and "High" corresponding to one SD below and above the mean, respectively (see text for explanation of procedure). "+" indicates dilation and "-" indicates constriction.

	HDL (Low)	HDL (High)
MAP change (Low)	+0.45%	-0.34%
MAP change (High)	-0.23%	-0.10%

FIGURE 1

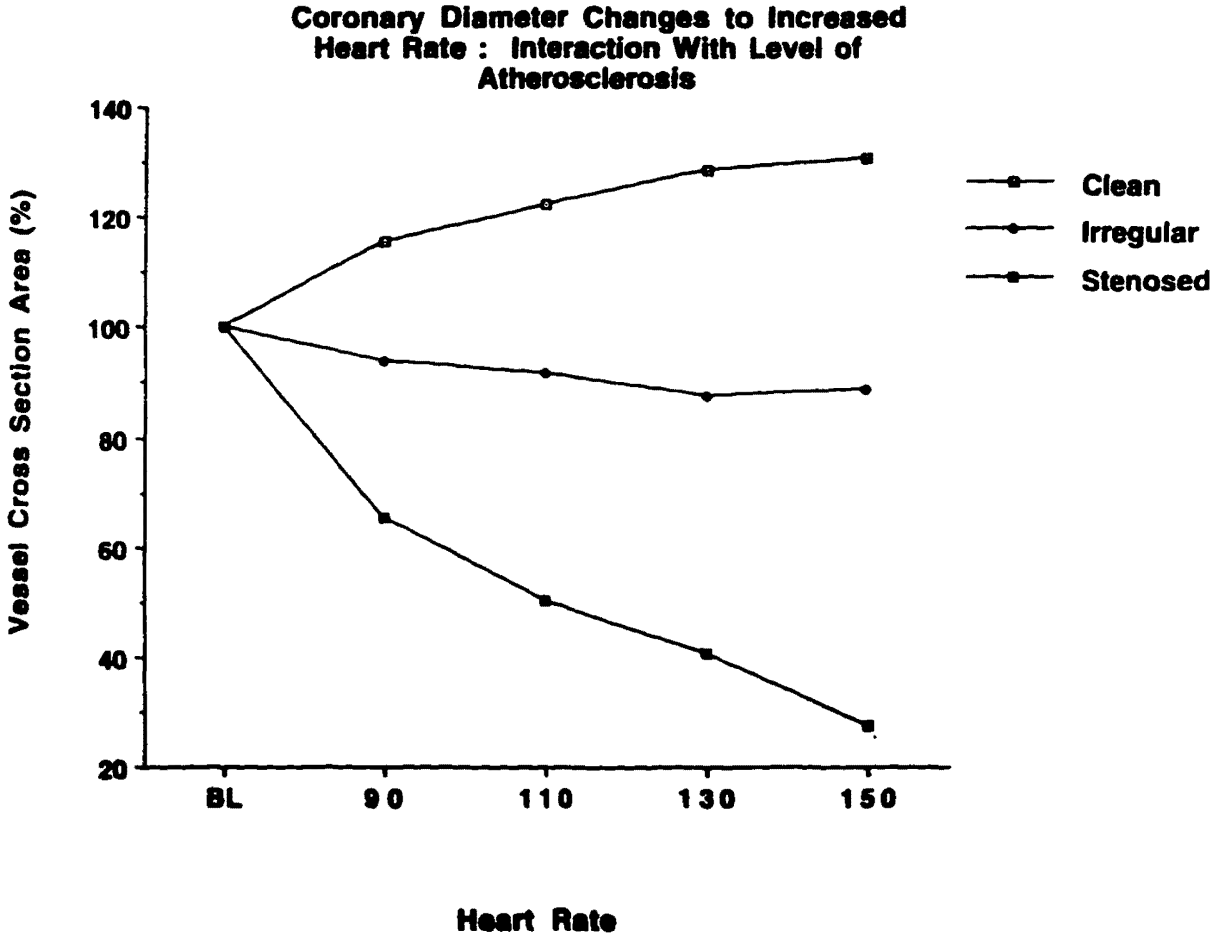

Coronary Diameter Changes to Increased Heart Rate : Interaction With Level of Atherosclerosis
Vessel Cross Section Area (%)
140
120
100
80
60
40
20
BL
90
110
130
150
Heart Rate
Clean
Irregular
Stenosed

FIGURE 2

Scatterplot of Percent Change in Atherosclerotic Vessel Diameter From Baseline to Mental Stress by Mean Arterial Pressure Response to Mental Stress

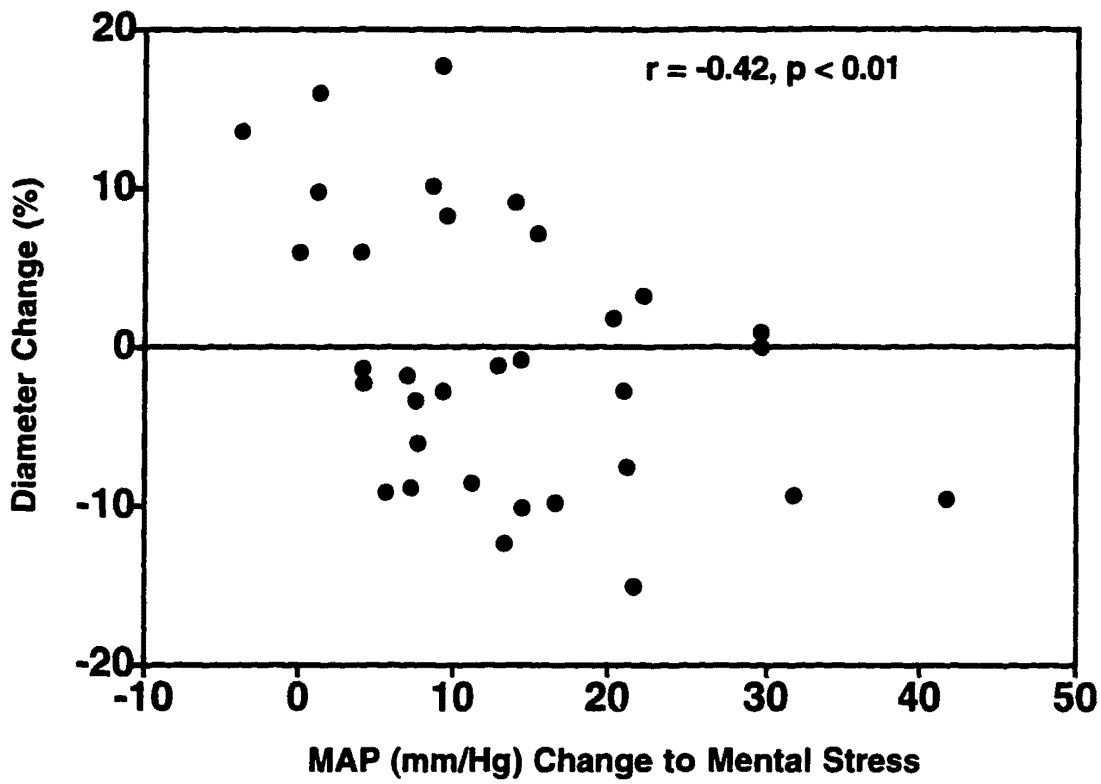


FIGURE 3

Mean Percent Change in Coronary Diameter to Mental Stress: High and Low Mean Arterial Blood Pressure (MAP) Reactors by Type of Coronary Segment (Atherosclerotic vs. Smooth)

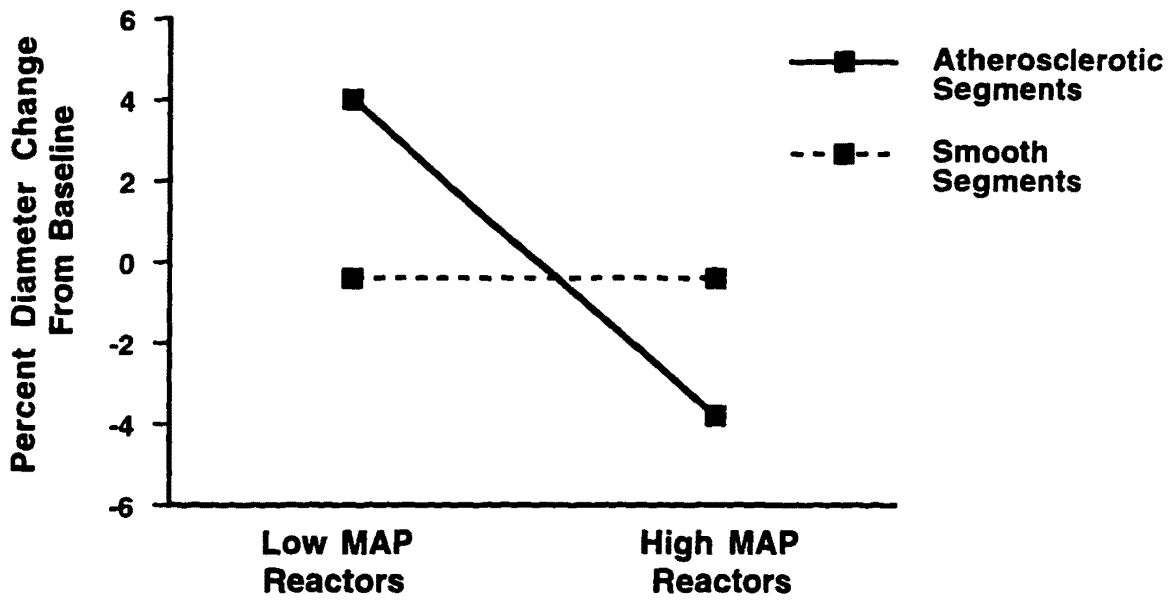


FIGURE 4

Mean Percent Change in Coronary Diameter to Mental Stress: High and Low Heart Rate (HR) Reactors by Type of Coronary Segment (Atherosclerotic vs. Smooth)

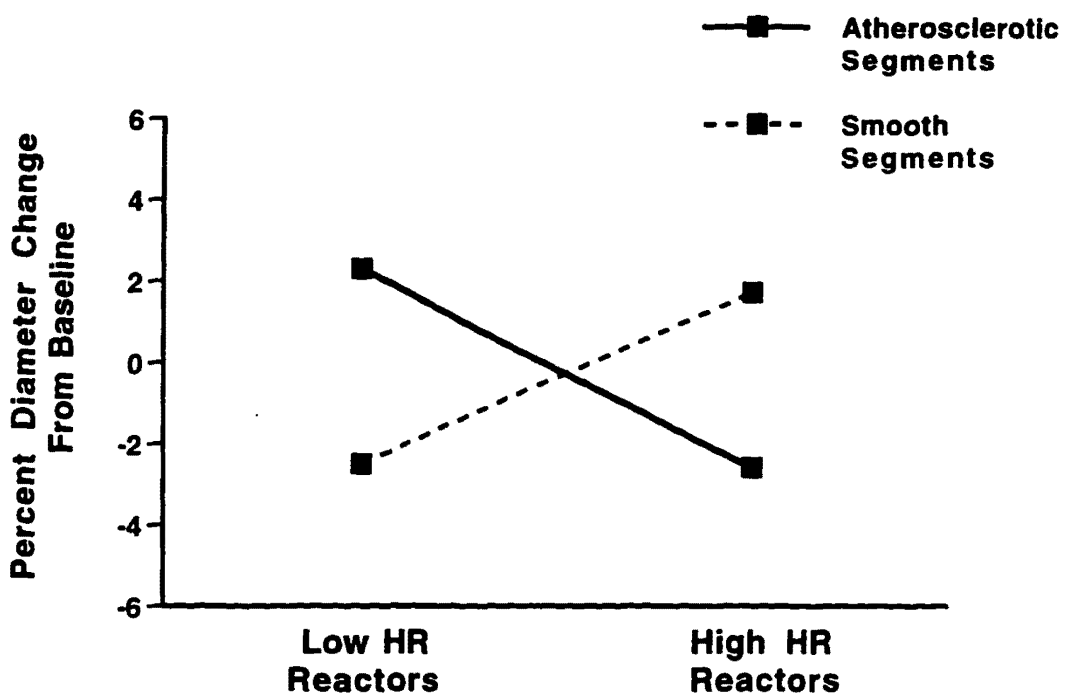
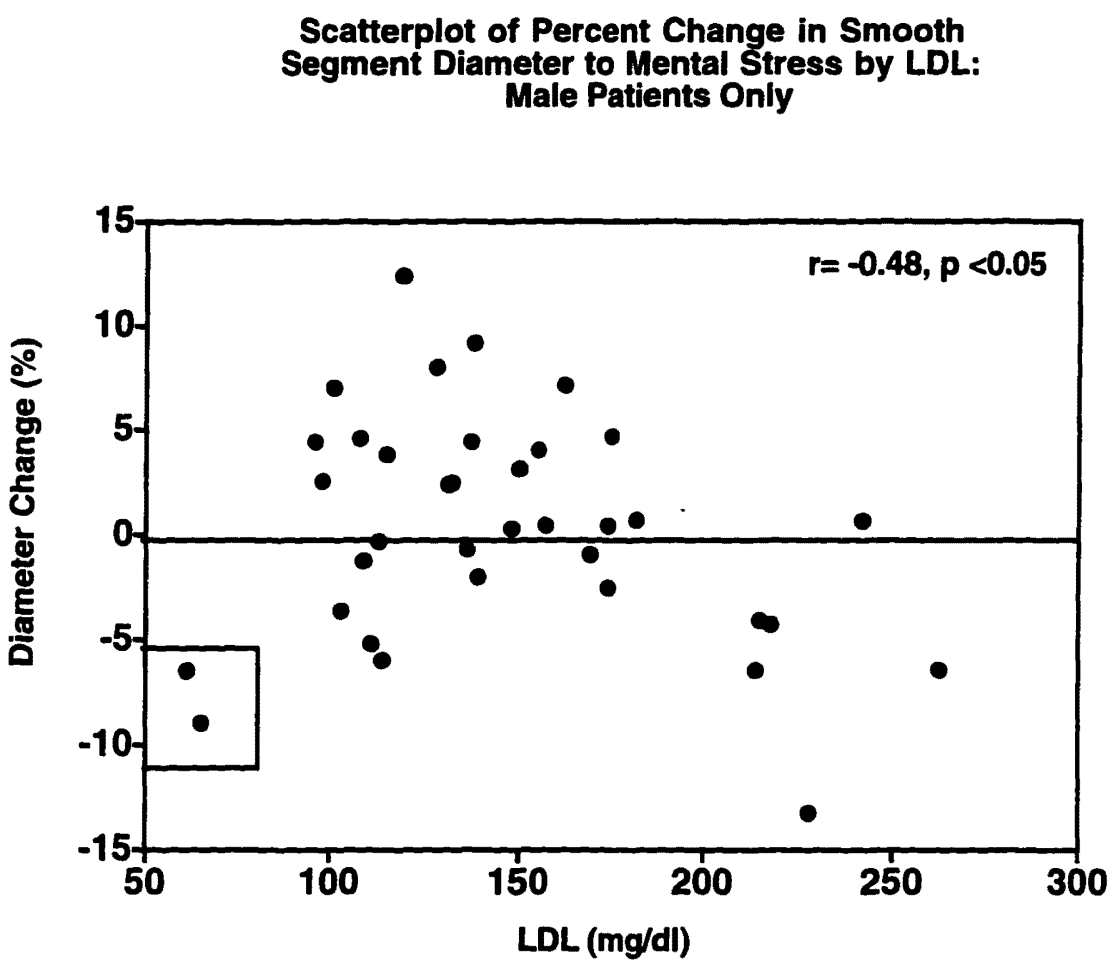


FIGURE 5


Scatterplot of Percent Change in Smooth Segment Diameter to Mental Stress by LDL: Male Patients Only
r= -0.48, p <0.05
Diameter Change (%)
15
10
5
0
-5
-10
-15
50
100
150
200
250
300
LDL (mg/dl)

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